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(54) Title: INHIBITION OF PRIMARY AND/OR SECONDARY IMMUNE RESPONSE TO REPEAT ADENOVIRAL VECTOR ADMINISTRATION USING CD40L SPECIFIC ANTIBODIES

(57) Abstract

A method is provided for decreasing or eliminating an immune response to the administration of an adenoviral vector containing a transgene to an individual comprising co-administering to said individual an amount of an antibody specific for CD40L on CD4+ T lymphocytes such that primary and/or secondary immune responses to the adenoviral vector are diminished or eliminated and expression of the transgene persists.

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Description

INHIBITION OF PRIMARY AND/OR SECONDARY IMMUNE RESPONSE TO REPEAT ADENOVIRAL VECTOR ADMINISTRATION USING CD40L SPECIFIC ANTIBODIES

Field of the Invention

The present invention relates to the use of antibodies specific for a helper T cell (T_h) ligand (CD40L), that binds to a receptor (CD40) that is found on B cells and other anigen presenting cells (APCs) and which is required for their activation. The antibodies of the invention are useful in reducing the host immune response to recombinant Adenoviral (Ad) vectors carrying a therapeutic transgene, thereby allowing effective repeat administration of such Ad vectors to a host.

Background of the Invention

E1-deleted replication-defective adenoviral (Ad) vectors are attractive vehicles for gene transfer to host cells because of their ability to transduce a wide variety of dividing and non-dividing cells *in vivo* (Stratford-Perricaudet et al., Hum. Gene Ther. 1:241-256 (1990); Rosenfeld et al., Cell 68:143-155 (1992); Zabner et al., Cell 75:207-216 (1993); Crystal et al., Nat. Genetics 8:42-51 (1994); Zabner et al., Nat. Genetics 6:75-83 (1994)). Such vectors have been used for transfer of the gene encoding normal human cystic fibrosis transmembrane conductance regulator (CFTR) into airway epithelial cells of experimental animals (e.g. mice, cotton rats, monkeys) and to airway epithelium of individuals with cystic fibrosis (CF) (Rosenfeld et al., Cell 68:143-155 (1992); Zabner et al., Cell 75:207-216 (1993); Crystal et al., Nat. Genetics 8:42-51 (1994); Zabner et al., Nat. Genetics 6:75-83 (1994)). Such vectors have transiently produced normal chloride ion channel function in CF patient airway epithelial cells.

A number of studies, however, have suggested that administration of high doses of first generation Ad vectors results in only transient CFTR gene expression in the lung due, at least in part, to destruction of vector-transduced cells by host cellular immune

responses (predominantly CD8[+] cytotoxic T cells) directed against Ad viral proteins and/or immunogenic transgene products (Yang et al., J. Virol. 69, 2004-2015 (1995); Kaplan et al., Gene Ther. 3:117-127 (1996); Tripathy et al., Nat. Medicine 2:545-550 (1996); Yang et al., Gene Ther. 3:137-144 (1996)). Reduction of this adverse immune response has been reported with the use of second generation vectors having decreased viral gene expression (Yang et al., Nature Genet. 7:362-369 (1994); Engelhardt et al., Proc. Natl. Acad. Sci. USA 91:6196-6200 (1994)) and with transgenes encoding self rather than foreign proteins (Tripathy et al., Nat. Medicine 2:545-550 (1996)).

The treatment of chronic diseases like CF with Ad vectors will likely require repeated administrations of Ad vectors containing the CFTR gene throughout the lifetime of the patient. However, as noted, the effectiveness of current Ad vectors is limited by the difficulty in obtaining successful readministration to an individual using a vector of the same Ad serotype, because of adverse immunologic responses. Various groups have demonstrated that a strong dose-dependent humoral immune response is induced by Ad vectors leading to the development of Ad-specific neutralizing antibodies, which leads to the inactivation by the host of readministered vector. (Yang et al., J. Virol. 69:2004-2015 (1995); Kaplan et al., Gene Ther. 3:117-127 (1996); Smith et al., Gene Ther. 5:397-402 (1993); Yei et al., Gene Ther. 1:192-200 (1994); Van Ginkel et al., Human Gene Ther. 6:895-903 (1995); Mastrangeli et al., Hum. Gene Ther. 7:79-87 (1996)). Studies using immunodeficient mice have shown that this process is dependent on MHC class II presentation of the input viral proteins and activation of CD4[+] T (helper) cells and can be induced by inactive as well as active viral particles (Yang et al., J. Virol. 69:2004-2015 (1995)).

In order to overcome the immunologic problems associated with repeat administration of Ad vectors, the use of broad immunosuppressants (Engelhardt et al., Proc. Natl. Acad. Sci. USA 91:6196-6200 (1994)) and cytoablative agents (Dai et al., Proc. Natl. Acad. Sci. USA 92:1401-1405 (1995)) to overcome the immune response of the host to first generation Ad vectors have been tested. Transient co-administration of an immunoglobulin, CTLA4-Ig, along with an intravenous injection of Ad vector

expressing a nonimmunogenic transgene product (human α-1 anti-trypsin) has been shown to lead to persistent transgene expression from mouse liver (Kay et al., Nat. Genetics 11:191-197 (1995)). CTLA4-Ig blocks the B7-CD28 pathway of T cell co-stimulation, which is required for optional activation of T cells. (Jenkins et al., Immunity 1:443-446 (1994); Lenschow et al., Ann. Rev. Immunol. 14:233-258 (1996)). Although Ad-specific antibody levels were reduced in CTLA4-Ig treated mice, the inhibition was not sufficient to allow secondary gene transfer via repeat administration of the vector under the conditions tested (Kay et al., Nat. Genetics 11:191-197 (1995)).

Co-administration of interferon-γ (INF-γ) or interleukin-12 (IL-12) with recombinant Ad vectors was shown to diminish the formation of Ad-specific neutralizing antibodies and allowed readministration of the vector to mouse airways (Yang et al., Nat. Medicine 1:890-893 (1995)). However, IL-12 is a potent mediator which affects T_{h1}-type $CD4^+$ T cell responses and is involved in stimulating natural killer (NK) cells and promoting the differentiation of cytotoxic T cells (CTLs) (Paul et al., Cell 76:241-251 (1994); Trinchieri, G., Blood 84:4008-4027 (1994); Bliss et al., J. Immunol. 156:887-894 (1996)). INF-γ is known to upregulate MHC class I on antigen presenting cells (Yang et al., Proc. Natl. Acad. Sci. USA 92:7257-7261 (1995)). Thus, both INF-Y and IL-12, while capable of inhibiting humoral immunity, might enhance the elimination of Ad vector transduced cells by CTLS (enhanced T_{h1} response).

It is well known that activated T cells play a critical role in the generation of both humoral and cellular immune responses. The interaction between the T cell receptor (TCR) and antigen-major histocompatibility complex (MHC) expressed on the surface of an antigen presenting cell (APC) is necessary, but not sufficient for the optimal activation of T cells which also requires additional co-stimulatory signals provided by several receptor-ligand pairs including B7-CD28 and CD40-CD40 ligand (CD40L) (Lenschow et al., Ann. Rev. Immunol. 14:233-258 (1996)).

CD40 is a 50kd molecule that has been identified on the surface of immature and mature B lymphocytes which, when crosslinked by antibodies, induces B cell proliferation. (Valle et al., Eur. J. Immunol. 19:1463-1467 (1989); Godon et al., J.

Immunol. 140:1425-1430 (1988); Gruber et al., J. Immunol. 142:4144-4152 (1989)). CD40, which has been found on human as well as murine B cells and other APCs, has been molecularly cloned and characterized. (Stamenkovic et al., EMBO J. 8:1403-1410 (1989)). Such other APCs include, inter alia, macrophages, dendritic cells, langerhans cells, endothelial cells, basal epithelial cells and thymic cells. (See, e.g., Foy et al., Ann. Rev. Immunol. 14; 591-617 (1996)).

A ligand for CD40, gp39 (also called CD40 ligand or CD40L) has also been molecularly cloned and characterized. (Armitage et al.. Nature 357:80-82 (1992); Lederman et al., J. Exp. Med. 175:1091-1101 (1992); Hollenbaugh et al., EMBO J. 11:4313-4319 (1992)). CD40L (gp39) protein is expressed on activated, but not resting, $CD4^{+}$ T_h cells from humans and mice. (Spriggs et al., J. Exp. Med. 176:1543-1550 (1992); Lane et al., Eur. J. Immunol. 22:2573-2578 (1992); Roy et al., J. Immunol. 151:1-14 (1993)).

As noted, CD40L (gp39) has been shown to be expressed transiently at high levels on activated $CD4^{+}$ T cells (Noelle et al., Proc. Natl. Acad. Sci. USA 89:6550-6554 (1992); Foy et al., J. Exp. Med. 178:1567-1575 (1993)). The co-stimulation provided by CD40L on T cells interacting with CD40 on B cells and other APCs seems to be essential for thymus-dependent humoral immunity (Foy et al., J. Exp. Med. 178:1567-1575 (1993); Kawabe et al., Immunity 1:167-178 (1994); Xu et al., Immunity 1:423-431 (1994); Foy et al., Ann. Rev. Immunol. 14:591-617 (1996)). For example, cells transfected with the CD40L (gp39) gene and expressing CD40L on their surface can trigger B cell proliferation and, together with other stimulatory signals, can induce antibody production. (Armitage et al., Nature 375:80-82 (1992); Hollenbaugh et al., EMBO J. 11:4313-4319 (1992)). CD40L also appears to play an important role in the generation of cellular immune responses as a component of the cascade of events leading to the production of helper cytokines (Foy et al., Ann. Rev. Immunol. 14:591-617 (1996); Stuber et al., J. Exp. Med. 183:693-698 (1996); Stout et al., J. Immunol. 156:8-11 (1996)).

It has been shown that blockage of CD40L, e.g. with a specific antibody to CD40L can block the occurrence of chronic and acute forms of graft vs. host (GVH) disease (Durie et al., J. Clin. Invst. 94:1333-1338 (1994) and prevent collagen induced arthritis (Durie et al., Science 261:1328-1330 (1993). Likewise, published PCT application WO95/06666 discloses various anti-CD40L antibodies and their uses in mediating or inhibiting various helper T cell mediated immune responses. Moreover, transient subversion of CD40L by a specific antibody to CD40L has been shown to diminish primary immune responses to Ad vectors in mouse liver and lung tissues (Yang et al., J. Virol 70:6370-6377 (1996)).

Summary of the Invention

The present invention is directed to reducing the immune response of a host to administered adenoviral (Ad) vectors carrying a therapeutic transgene, thereby allowing for repeat readministration of such vectors to the host without (or with minimized) adverse immune responses.

The present invention is of particular relevance since Ad vectors are especially attractive for use in delivering a therapeutic transgene to host cells, e.g., in gene therapy, based in part on their ability to efficiently transfer the transgene into host cells, particularly non-dividing cells, in vivo. However, significant immune response to such Ad vectors, inflammation and loss of transgene expression has limited the effective use of Ad vectors in gene transfer and therapy.

The immune response to Ad vectors appears to be mediated through activation of $CD4^+$ T cells by viral antigens leading to $CD8^+$ cytotoxic T lymphocyte (CTL) and humoral immune responses to the vector. The CD40 ligand (CD40L) on T cells and CD40 receptors on B cells and other APCs are involved in generating the adverse immune response to administered Ad vectors.

The present invention thus provides for co-administration of monoclonal antibodies (MAbs) specific for CD40L together with recombinant Ad vectors in order to

minimize or ablate the host immune response to the administered Ad vector, thereby allowing for repeat administration of such vectors and persistent transgene expression.

Specifically, in a mouse model system, MAb to murine CD40L provided for transient blockade of co-stimulation between activated T cells ($CD4^+$) and B cells/antigen presenting cells (APCs) and inhibited neutralizing antibodies and the cellular response to a co-administered Ad vector. Co-administered anti-CD40L MAb also provided for increased persistence of expression of the transgene delivered to host cells by the Ad vector.

In a further aspect of the invention, the administration of anti-CD40L MAb interfered with secondary immune (antibody) responses in a preimmunized host to readministered Ad vectors, thereby allowing for repeat administrations of the vector, with high levels of transgene expression. This is highly significant, because most human recipients of Ad vector - based gene therapy are expected to have prior exposure to human adenoviruses and, therefore, to have an immunological memory directed to adenovial antigens.

In a still further aspect of the invention, the anti-CD40L MAb is a specific MAb designated MR1, which is a hamster-derived anti-murine CD40L MAb. The expressed transgene is the DNA for CFTR or β-galactosidase.

Brief Description of the Drawings

The present invention may be further understood with reference to the attached drawings, of which

Fig. 1 shows the effect of MR1 on the development of Ad-specific antibodies following administration of Ad2/CFTR2 vector. Panel A shows Ad-specific serum antibodies. Panel B shows Ad-specific IgA levels in the lung.

Fig. 2 shows the effect of MR1 on efficient readministration of a second dose of an Ad vector. The graphs show β-galactosidase expression in the presence and absence of administered MR1.

Fig. 3 depicts the effect of MR1 on CTL responses in mice intranasally administered Ad2/βGal4.

Fig. 4 shows the effect of MR1 on persistence of transgene (β-gal) expression in mice.

Fig. 5 shows decreased lung inflammation to Ad2/CFTR2 in MR1-treated mice.

Fig. 6 shows the effect of MR1 administration on the secondary immune response to Ad vectors.

Fig. 7 shows the effect of MR1 administration on Ad-specific antibody neutralizing titers upon readministration of Ad vectors.

Fig. 8 shows the effects of MR1 administration on transgene expression (β-gal) upon a third administration of Ad vector.

Detailed Description of the Invention

The present invention is directed to diminishing or inhibiting adverse immunologic responses in a host individual to an administered Adenoviral (Ad) vector comprising a therapeutic transgene. The invention involves co-administering to said host individual an effective amount of an inhibitor for CD40L on $CD4^+$T cells, particularly an antibody, and more particularly a monoclonal antibody (MAb) specific for CD40L. The dosage, timing, and routes of administering the Ad vector and CD40L specific MAb to the host is chosen to be most effective in minimizing or inhibiting the primary and secondary humoral and cellular immune responses in the host to the administered vector. Thus, Ad-specific neutralizing antibodies, CTLs and inflammatory responses are reduced. The administration of CD40L specific antibodies allows for repeat administration of the same adenoviral vectors to the host, with minimized adverse immunological consequences and persistent transgene expression in treated host cells.

In preferred aspects of the invention, the adenoviral (Ad) vector is an Ad 2 vector having a substantially deleted E1 region and E4 region (except for open reading frame 6). The vector further comprises a therapeutic transgene operably linked to expression control sequences (promoter, poly A-tail) inserted into the deleted E1 region of the

vector. In a preferred example, the vector is Ad2/CFTR2 which contains the DNA coding for cystic fibrosis transmembrane regulator and can, upon administration to airway epithelial cells of CF patients, result in functional chloride ion channel activity in such cells. (Zabner et al., J. Clin. Invest. 97:1504-1511 (1996)).

Other vectors useful in the present invention include Ad2/β-Gal2 and Ad2/β-Gal4. These latter Ad vectors express β-gal in host cells which can be used as a marker for transgene expression. Other Ad vectors comprising additional therapeutic transgenes are within the scope of the invention.

In other aspects of the invention, the effectiveness of the approach to reducing adverse immune responses to Ad vectors is demonstrated in a standard mouse model system, particularly Balb/c mice. In particular, the Ad vectors used in the invention are intranasally administered to the mice, resulting in Ad infection of host airway epithelial cells (e.g. in the lung).

In order to reduce or inhibit primary and secondary immune responses to the administered Ad vector and provide for effective readministration of the vector and persistent transgene expression, CD40L on $CD4^+T$ cells was blockaded by co-administration of anti-murine CD40L MAb, particularly the hamster-derived MR1 antibody. While whole purified MR1 antibody was used, it is expected that fragments of such antibodies, e.g. $(Fab')_2$, Fab, Fv and others should also be useful in producing the desired response.

Moreover, while effectiveness of the approach to reducing the immune response to Ad vectors via inhibiting CD40L has been shown in a mouse model system, it is expected that such approaches also work in humans, particularly CF patients. It has already been demonstrated that CFTR containing Ad vectors can produce functional chloride channels in airway epithelium in CF patients. The co-administration of anti-human CD40L antibodies can reduce or inhibit the adverse immune responses believed to be the primary reason for reduced effectiveness of repeated CFTR-containing Ad vector administration for treating CF patients.

The anti-human CD40L antibodies for inhibiting inmmune responses to Ad vectors in humans can be either polyclononal or monocional antibodies (MAbs). Such antibodies can be of animal origin such as rabbit or rodent MAbs. The anti-human DC40L antibodies can be of human origin. Such antibodies also can be of animal origin. e.g. rodent MAbs, that have been "humanized" using techniques known by those skilled in the art.

Example 1

Anti CD40L Monoclonal Antibodies

Anti-CD40L MAbs may be made as known in the art. A detailed recitation of methods for producing such antibodies is provided in published PCT application WO95/06666, incorporated herein by reference. Techniques can be used to produce such MAbs in mice, hamsters and rabbits. Likewise, human MAbs to CD40L may be obtained as described in WO95/06666.

MR1, a hamster anti-mouse CD40L was produced in ascites fluid and purified by ion exchange HPLC as described by Noelle et al., Proc. Natl. Acad. Sci. USA 89:6550-6554 (1992), incorporated herein by reference.

Other antibodies contemplated for use in the present invention include anti-human CD40L MAbs of murine origin as disclosed in WO95/06666 (Example 6). Such antibodies include, inter alia, the hybridomas designated 89-76 and 24-31 that have been deposited with the ATCC.

However, for administration of anti CD40L antibodies to humans, it may be preferable to use antibodies of human origin or those that have been "humanized" to avoid or reduce potential adverse immunological responses to rodent MAbs (e.g. mouse or hamster). It is believed, however, that any such potential adverse effects should be minimal, since the reason for administering antibodies to CD40L is to reduce or inhibit immune responses to a foreign antigen. Techniques known to those of skill in the art, e.g., recombinant technology may be used to construct "humanized" anti-CD40L antibodies that maintain the high binding affinity of the rodent MAbs.

For example, recent approaches to humanizing rodent MAbs use only the rodent complementarity determining regions (CDRs), rather than the entire V domain, transplanted to a human antibody. Such humanized antibodies are known as CDR-grafted antibodies. CDRs are regions of hypervariability in the V regions that are flanked by relatively conserved regions known as framework (FR) regions. Each V domain contains three CDRs flanked by four FRs. The CDRs fold to form the antigen binding site of the antibody, while the FRs support the structural conformations of the V domains. Thus by transplanting the rodent CDRs to a human antibody, the antigen binding domain theoretically also is transferred. (Owens et al. J. Immunol. Methods 168:149 (1994) and Winter et al. Immunology Today 14:243 (1993) incorporated herein by reference.)

Different avidities of humanized MAbs also appear to depend upon the particular human framework region (FR) of the humanized antibody. For example, Co et al. Proc. Natl. Acad. Sci. USA 88:2869 (1991) required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. Protein Engineering 4:773 (1991) reported the influence of particular FR residues of a CDR-grafted antibody on antigen binding, and proposed that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. J. Immunol. 150:2844 (1993) reported that optimal humanization of an anti-CD18 murine monoclonal antibody was dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation.

Accordingly, it will be apparent to those skilled in the art that recreation of the antigen-binding site requires consideration of the potential intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see e.g., Owens et al.), in all cases the procedures must be tailored and optimized for the particular rodent antibody of interest.

Example 2

Antibody Injections

Balb/c mice, purchased from Taconic Laboratories (Germantown, NY) were used as the model system for assessing the effectiveness of anti-CD40L MAbs in reducing immune responses to administered Ad vectors.

Typically, mice were injected intraperitoneally (IP) with a total of four injections each of 200-250 μg purified antibody (MR1) starting on day -2 relative to the time of administering the Ad vectors.

Example 3

Adenoviral Vectors

The construction of Ad2/CFTR2 has been described in detail in allowed U.S. Patent Application Serial No. 08/136,742 and in Armentano et al. (1995) Human Gene Ther. 6:1353, both incorporated herein by reference.

Ad2/CFTR2, is an Ad2 based vector in which substantially all of the adenoviral E1 region has been deleted and replaced with a CFTR transgene expression cassette comprising a PGK promoter, the CFTR encoding sequence and a BGH poly A site and most of the E4 region has been deleted, except for open reading frame 6 (ORF-6).

Administration of Ad2CFTR2 to nasal epithelia of CF patients has resulted in restoration of a functional chloride ion channel in treated cells. Zabner et al. J. Clin. Invst. 97:1504-1511 (1996) incorporated herein by reference. However, adverse host immune responses limited the gene transfer effectiveness.

Ad2/βGal2 is derived from Ad2/CFTR2, but with the CFTR expression cassette replaced by a DNA fragment comprising the CMV promoter, the lacZ(β-galactosidase encoding) gene and a 5' nuclear localization signal from the SV40T antigen.

Ad2/βGal4 is similar to Ad2/βGal2 but it contains a complete wild type E4 region rather than just ORF6.

Example 4

Histopathology of Mouse Lung Tissue Following Ad Vector Administration

On the day of sacrifice, mice were euthanized with an IP injection of Somlethal. The lungs were cleared of blood by vascular perfusion with phosphate buffered saline (PBS). The trachea was cannulated and the lungs and trachea removed. The lungs were fixed by inflation with 2% paraformaldehyde containing 0.2% glutaraldehyde in PBS, pH 7.4, at a pressure of 30 cm of H_20. Following overnight fixation, portions of the left lung were embedded in glycomethacrylate, sectioned and stained with hematoxylin and eosin. These sections were evaluated by light microscopy for the presence and distribution of lung inflammation without previous knowledge of treatment as provided in Ginsberg et al. Proc. Natl. Acad. Sci. USA 88:1651-1655 (1991), incorporated herein by reference. The lung sections were subjectively assessed for morphologic alterations on a scale of 0-4: 0=no lesion, 1=minimal, 2=mild, 3=moderate, 4=severe.

Example 5

Measurement of Host Antibodies to Adenovirus

Titers of Ad-specific serum antibodies were evaluated by ELISA techniques as follows. Serial 2-fold dilutions of sample were added to the wells of a 96 well plate coated with photochemically inactivated Ad2 (Lee Biomolecular Research, San Diego, CA). Bound virus-specific antibodies were detected by the addition of horseradish peroxidase (HRP)-conjugated goat anti-mouse Ig (IgG, IgM, IgA-specific; Jackson Immunoresearch Laboratories, West Grove, PA). The titer was defined as the reciprocal of the highest dilution of sample which produced an $O.D._{490}$ greater than 0.1.

To evaluate levels of Ad-specific IgA in bronchoalveolar lavages (BAL), samples were diluted 2-fold and added to Ad2-coated plates followed by the addition of HRP-conjugated goat anti-mouse IgA (α chain-specific; Cappel, Durham, NC). For quantitation, a standard curve was constructed using a monoclonal antibody against mouse IgA (Harlan Sera-Lab, Sussex, England) to coat ELISA plates and capture known amounts of purified mouse IgA (Cappel). The $O.D._{490}$ values obtained following the

addition of HRP-conjugated goat antimouse IgA were plotted against the amounts of IgA standard (ng/ml) added to the wells. The concentrations of Ad-specific IgA present in BAL samples were then derived from the standard curve by linear regression analysis.

Example 6

Evaluation of Cytoxic T Lymphocyte (CTL) Activity in Response to Adenoviral Vectors

To evaluate cytotoxic T lymphocyte (CTL) activity, spleen cells from animals in the same group were pooled and stimulated in vitro with mitomycin-C inactivated, syngeneic fibroblasts infected with Ad2/βGal-4 at a multiplicity of infection (M.O.I.) of 50 for 24 hr. Cells were cultured in 24-well plates containing 5×10^6 spleen cells and 6×10^4 stimulator fibroblasts per well in a 2 ml volume. The culture medium consisted of RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 5×10^{-5}M 2-mercaptoethanol, 20 mM HEPES buffer and 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT). Cytolytic activity was assayed after 5-7 days of culture.

Target fibroblasts were infected with Ad vector at an MOI of 100 for 48h and were treated with 100 U/ml recombinant mouse γ-interferon (Genzyme, Cambridge, MA) for approximately 24 hr before use to enhance MHC Class I expression and antigen presentation to effector CTLS. The fibroblasts were labeled with ^{51}Chromium (^{51}Cr; NEN) overnight (50 μCi/10^5 cells) and added to the wells of a round-bottom 96 well plate in a 100 μl volume (5×10^3 fibroblasts/well). Effector cells were added in a 100 μl volume at various effector:target cell ratios in triplicate. After 5 hours of incubation at 37°C/5% CO_2, 100 μl of cell-free supernatant was collected from each well and counted in a Packard (Downers Grove, IL) Multi-Prias gamma counter. The amount of ^{51}Cr spontaneously released was obtained by incubating target fibroblasts in medium alone and the total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water. The percentage lysis was calculated as follows:

$$\% \text{ Lysis} = \frac{(\text{Sample cpm}) - (\text{Spontaneous cpm})}{(\text{Total cpm}) - (\text{Spontaneous cpm})} \times 100$$

Example 7

Measurement of β-Galactosidase Expression

For quantitation of β-gal expression, lungs from individual mice were homogenized and assayed using the AMPGD kit obtained from Tropix, Bedford, MA. The protein concentration in an individual sample was determined using the BioRad DC reagent (BioRad, Hercules, CA) and the results are expressed as relative light units (RLU)/μg protein.

Example 8

Determination of Titers of Neutralizing Antibodies Against Adenoviral Vectors

To determine Ad specific neutralizing antibody titers, serial 2-fold dilutions of an antibody sample were incubated with live Ad2/CFTR-2 for 1 hour at 37°C/5% CO_2 in the wells of flat bottom 96 well plates. At the end of the incubation period, permissive 293 cells were added to the wells and the plates were incubated at 37°C/5% CO_2 for 72-96 hours. The assay was read when control 293 cells incubated alone reached ≥90% confluency. The neutralizing antibody titer was defined as the reciprocal of the highest dilution of sample that showed any detectable protection of 293 cells from cytopathic effects when compared to cells incubated with untreated virus or virus incubated with seronegative serum.

Example 9

Monoclonal Antibody Specific For CD40L Inhibits Development of Host Antibodies to Administered Adenoviral Vectors

To explore the role of the CD40-CD40L interaction in the generation of antibodies to Ad vectors, Balb/c mice were injected intraperitoneally with the anti-CD40L MAb MR1 (Example 1). 200 μg/injection/mouse of MR1 on days -2, +2, +6 and +10) was given (Example 2). Ad2/CFTR2 vector (10^9 I.U.) was instilled intranasally on

day 0. Ad specific antibodies were measured as in Example 5. Analysis of serum from these animals by ELISA showed a marked decrease in anti-Ad antibody (IgG + IgM + IgA) titers in MRI treated mice for up to 41 days. Fig. 1A is a graph plotting Ad specific serum antibody titers measured by ELISA. Closed circles (-•-) show Anti-Ad titers in mice treated with MR1; open squares (-□-) show those not treated with MR1. Each point represents the mean titer of groups of individual mice (n=4) ± standard error of the mean (SEM).

Analysis of bronchoalveolar lavage (BAL) fluid 40 days after administration of the vector analyzed by ELISA revealed a parallel drop in Ad specific IgA levels in MRI treated mice (Figure 1B). The data are mean IgA levels (n=3) ±SEM. (Fig. 1B).

On day 38 after the first administration of Ad2/CFTR2, a vector of the same serotype, Ad2/βGal2, was administered to the different groups of mice. Expression of β-galactosidase was measured by the quantitative assay of Example 7 on day 41. The data, which are presented as mean ± SEM (n=4) shows elevated β-galactosidase levels in MRI treated mice compared to control mice (Fig. 2).

Example 10

Monoclonal Antibody to CD40L Produces
A Decreased CTL Response to Administered
Adenoviral Vector and Results in Increased
Persistence of Transgene Expression

BALB/c mice were instilled intranasally with 10^9 IU of Ad2/βGal-4 on day 0 and injected with MRI (250 μg/mouse/injection) on days -2, +2, +5 and +8. The spleen cells were collected on day 21, re-stimulated in vitro with infected syngeneic fibroblasts and tested for cytolytic activity. The results shown in Fig. 3 are mean percent lysis from triplicate wells at various target:effector ratios. Spleen cells from MR1 treated mice showed decreased yet measurable levels of CTL activity compared to spleen cells from untreated control mice, albeit using an assay that is not strictly quantitative (Example 6).

Since CTLs have been implicated in loss of transgene expression, administration of MR1 with an Ad vector containing β-gal transgene was tested to determine whether

the MR1 MAb would give rise to prolonged transgene expression. As shown in Fig. 4, transgene expression measured by the quantitative galactosidase assay of Example 7, declined to background levels by day 21 in the untreated controls. By comparison, in MR1 treated mice, transgene expression also declined but remained consistently higher than in untreated mice. The data are presented as mean ±SEM(n=4).

Example 11

Monoclonal Antibody to CD40L Results in
Decreased Inflammatory Responses to Adenoviral Vectors

Lung tissue from the mice of Example 9 was examined as provided in Example 4 for evidence of histopathological changes in the peribronchial, perivascular and alveolar regions. Lung inflammation characterized by inflammatory cell infiltrates was present on day 5 and was not yet resolved at day 21. On day 5, there were no differences noted between the lungs of mice treated with the vector either with or without MR1 antibody treatment (data not shown). However, as shown in Fig. 5, on day 21, there were fewer inflammatory changes in all regions of the lungs from mice treated with the MR1 antibody. The inflammatory cell infiltrate was markedly reduced in the peribronchial/peribronchiolar and perivascular regions.

Example 12

Monoclonal Antibody to CD40L Inhibits
Secondary Antibody Response to Adenoviral
Vectors in Preimmunized Hosts

MR1 was tested for its ability to interfere with the secondary antibody response in mice that had been preimmunized with an Ad vector. Mice were intranasally instilled with 10^8 IU of Ad2/CFTR2 on day 0. On day 50, 10^8 IU of Ad2/CFTR2 was intranasally administered to the same mice. It had previously been determined that an intranasal instillation of 10^8 IU of Ad vector elicits both humoral and cellular immune responses to the vector in several strains of mice including BALB/c (data not shown). MR1 injections were given around the time of the second virus administration on days 44, 48, 52 and 56.

Sera from the different groups of animals were analyzed both for anti-Ad ELISA titers (IgG + IgM + IgA) and Ad neutralizing titers at various time points (as provided in Example 5). Fig. 6 shows that the secondary antibody response to Ad measured by ELISA (serial 2-fold dilutions of sera obtained at the indicated times) was quite robust in both MR1-treated and -untreated mice and was only slightly decreased in the MR1-treated mice.

Ad neutralizing titers, on the other hand, were clearly decreased in the mice that had received MR1 along with the second administration of the Ad vector (Fig. 7). In addition, the decline in anti-Ad titers, after reaching a peak around day 84, was more rapid in MR1 treated mice compared to untreated mice (Fig. 6).

Example 13

Effect of Monoclonal Antibody to CD40L
On Third Administration of Adenoviral Vectors

A third intranasal administration of Ad2/βGal-2 vector was given on day 99 and β-galactosidase levels in the lung were measured on day 102. Control mice that had received the first (day 0) and second dose (day 50) of Ad2/CFTR2 showed only 6% of the β-galactosidase activity in lung homogenates compared to naive mice that received a single intranasal administration of Ad2/βGal-2 (Fig. 8). Mice that received MR1 around the time of the second administration of Ad2/CFTR2 showed 10-fold higher levels of transgene expression after the third administration compared to untreated control mice (Fig. 8). The effectiveness of the third vector administration in the MR1 treated animals was comparable to that obtained in naive mice.

Claims

1. A method for decreasing or eliminating an immune response to the administration of an adenoviral vector containing a transgene to an individual comprising co-administering to said individual an amount of an antibody specific for CD40L on CD4+ T lymphocytes such that primary and/or secondary immune responses to the adenoviral vector are diminished or eliminated and expression of the transgene persists.

2. The method of Claim 1, in which the antibody is a monoclonal antibody.

3. The method of Claim 2, in which the monoclonal antibody is MR1.

4. The method of Claim 2, in which the monoclonal antibody is selected from the group consisting of hybridoma 89-76 and hybridoma 24-31.

5. The method of Claim 1, in which the antibody is a fragment of an antibody.

6. The method of Claim 1, in which the antibody is a humanized antibody.

7. The method of Claim 1, in which the adenoviral vector is Ad2/CFTR2.

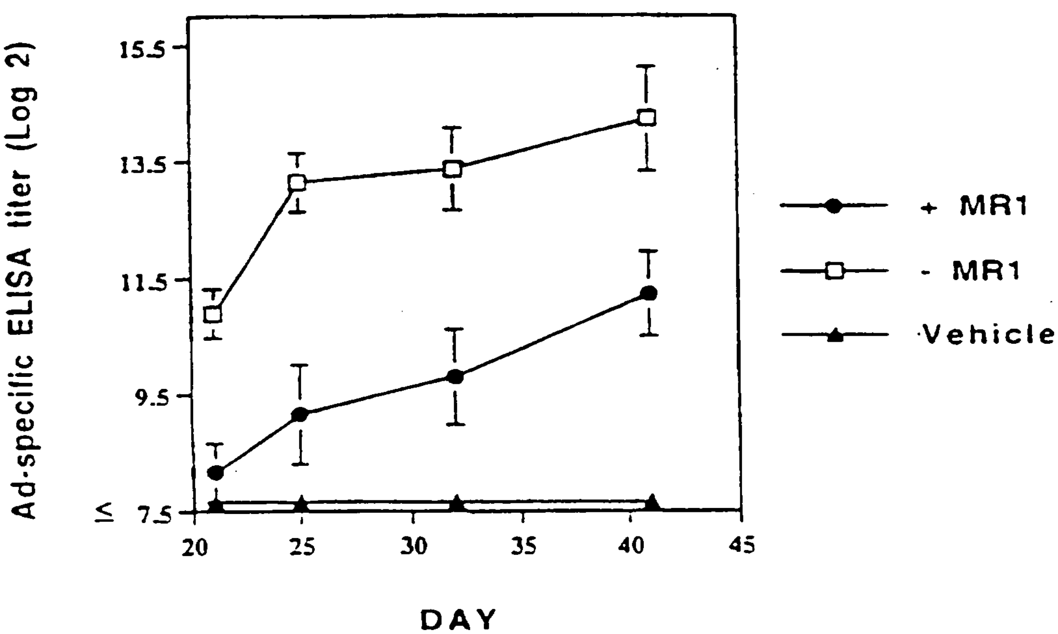

Ad-specific ELISA titer (Log 2)
15.5
13.5
11.5
9.5
≤ 7.5
20
25
30
35
40
45
DAY
+ MR1
- MR1
Vehicle

Figure 1A

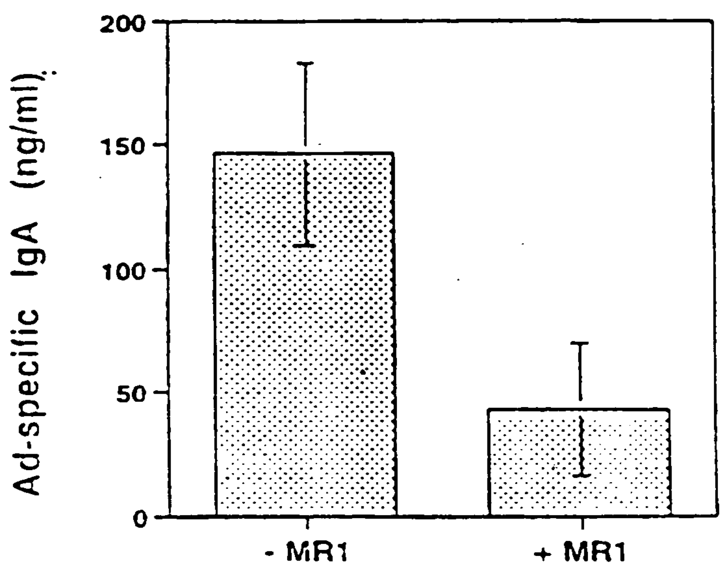


Figure 1B

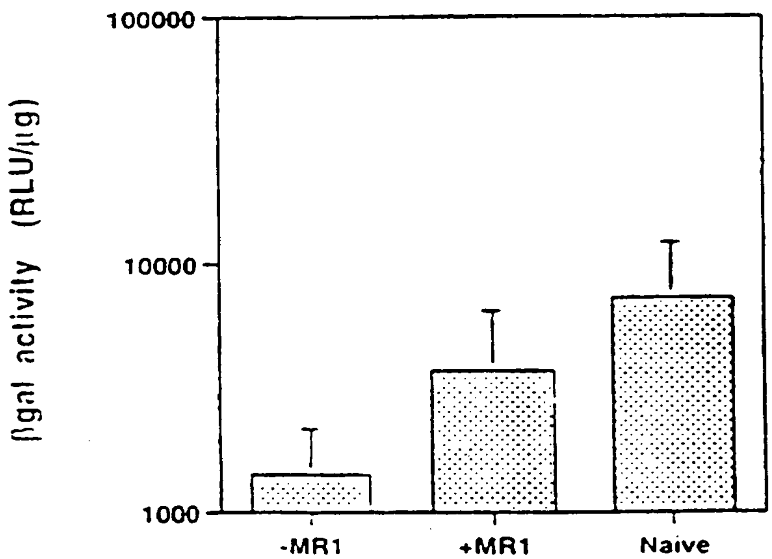


Figure 2

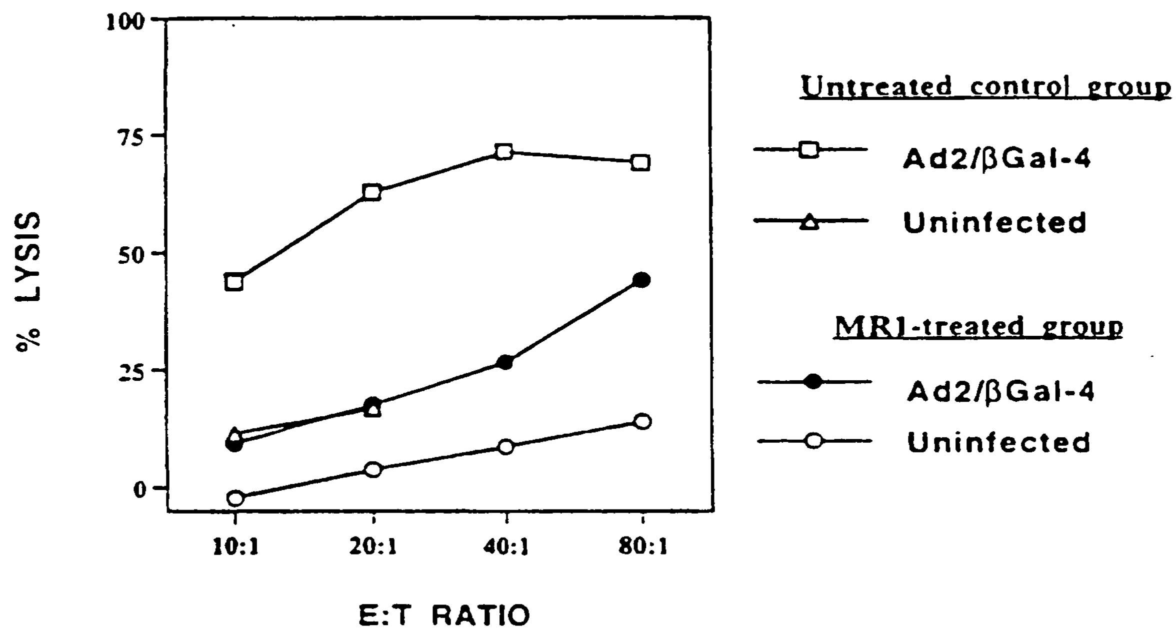

100
75
50
25
0
% LYSIS
10:1
20:1
40:1
80:1
E:T RATIO
Untreated control group
Ad2/βGal-4
Uninfected
MR1-treated group
Ad2/βGal-4
Uninfected

Figure 3

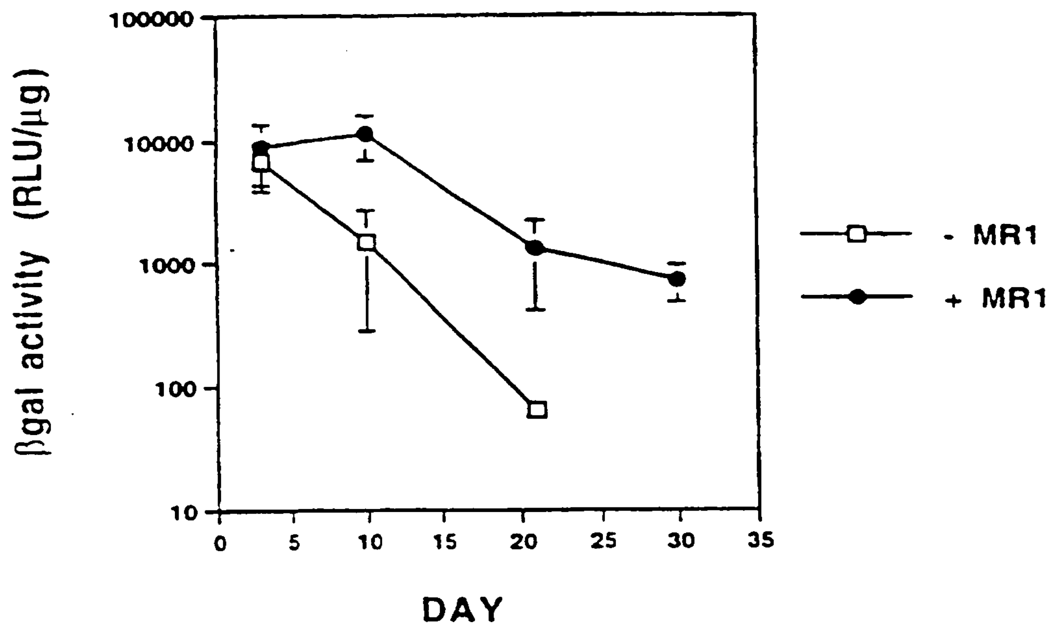

100000
10000
1000
100
10
βgal activity (RLU/μg)
0
5
10
15
20
25
30
35
DAY
- MR1
+ MR1

Figure 4

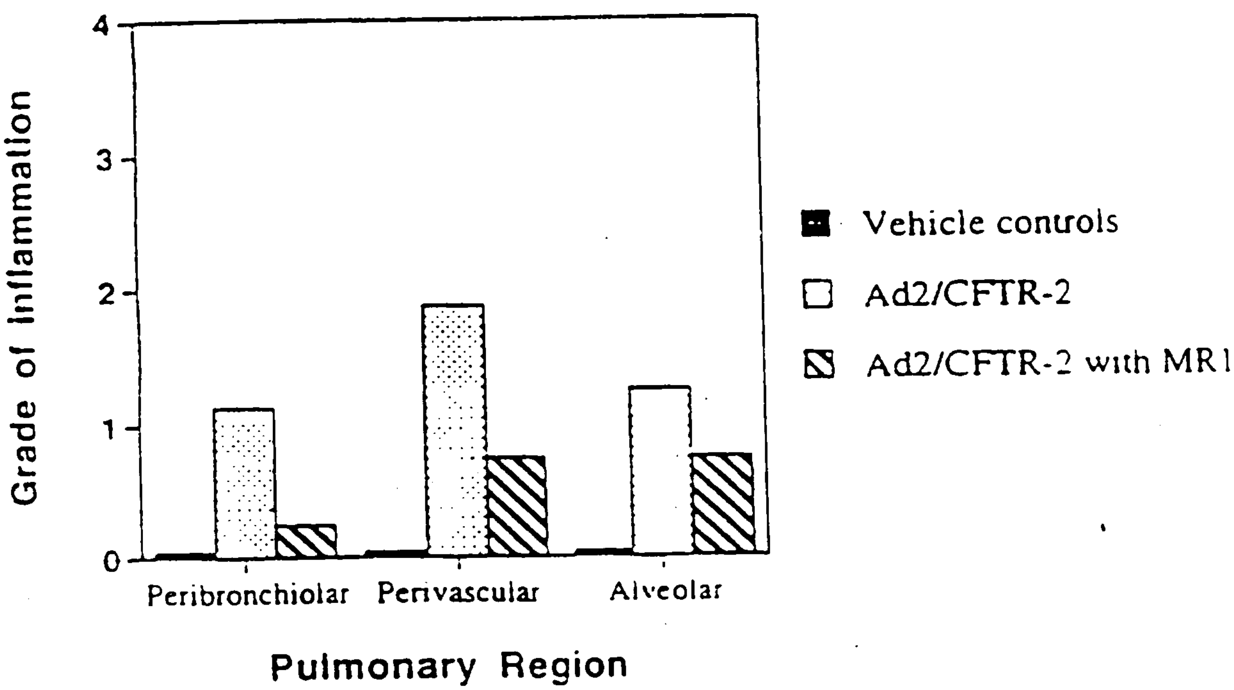

Grade of Inflammation
4
3
2
1
0
Vehicle controls
Ad2/CFTR-2
Ad2/CFTR-2 with MR1
Peribronchiolar
Perivascular
Alveolar
Pulmonary Region

Figure 5

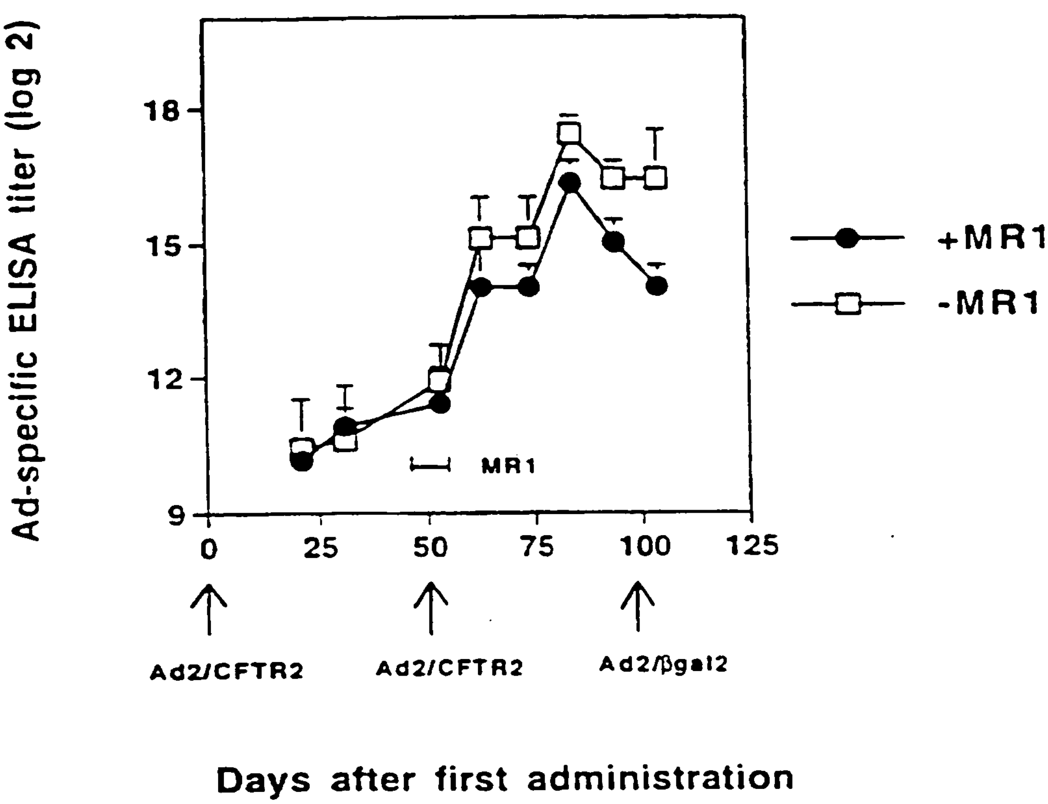

Ad-specific ELISA titer (log 2)
18
15
12
9
0
25
50
75
100
125
MR1
+MR1
-MR1
Ad2/CFTR2
Ad2/CFTR2
Ad2/βgal2
Days after first administration

Figure 6

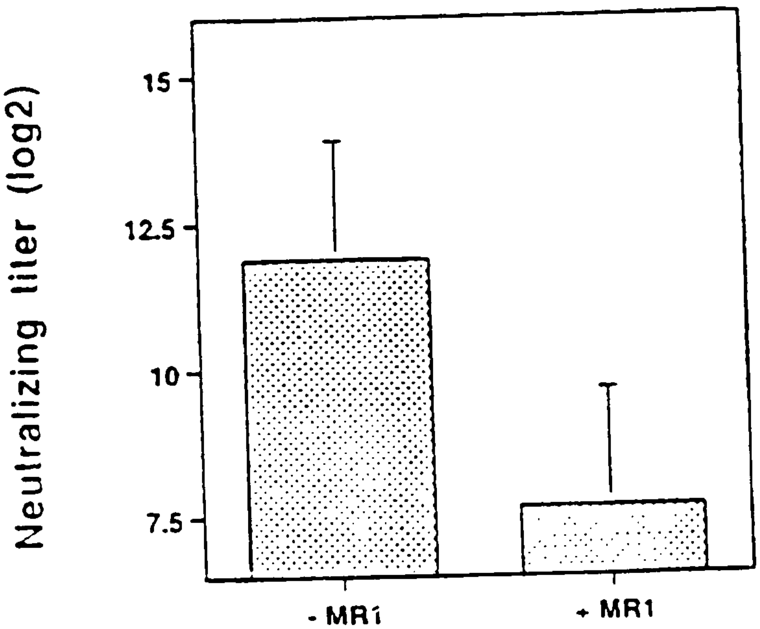
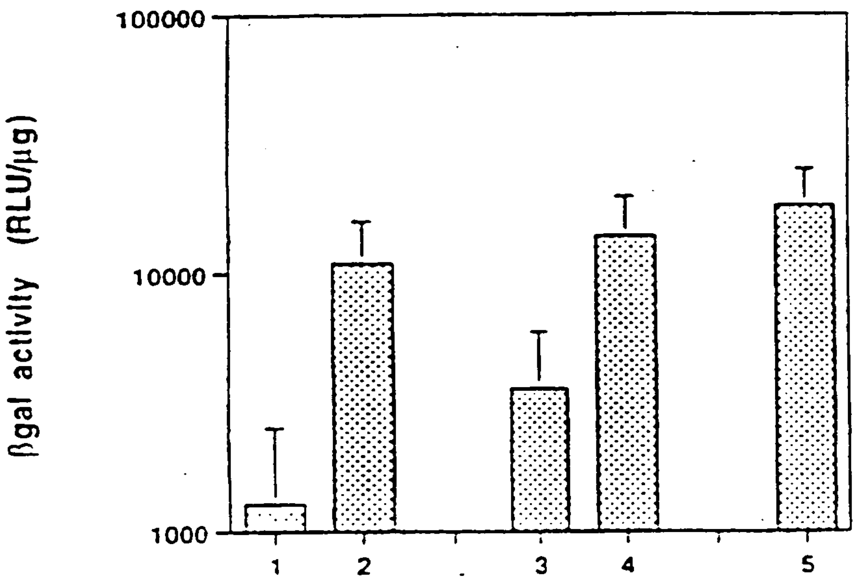


Figure 7



Group	Day 0	Day 50	Day 99
1	Ad2/CFTR2	Ad2/CFTR2	Ad2/βGal2
2	Ad2/CFTR2	Ad2/CFTR2 + MR1	Ad2/βGal2
3	-	Ad2/CFTR2	Ad2/βGal2
4	-	Ad2/CFTR2 + MR1	Ad2/βGal2
5	-	-	Ad2/βGal2

Figur 8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16487

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 C07K16/28 //A61K48/00,C12N15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DURIE F H ET AL: "PREVENTION OF COLLAGEN-INDUCED ARTHRITIS WITH AN ANTIBODY TO GP39, THE LIGAND FOR CD40" SCIENCE, vol. 261, no. 5126, 3 September 1993, pages 1328-1330, XP002035702 cited in the application see the whole document ---	1-7
Y	KAY M A ET AL: "Long-term hepatic adenovirus-mediated gene expression in mice following CTLA4Ig administration." NATURE GENETICS, vol. 11, no. 2, October 1995, UNITED STATES, pages 191-197, XP000676074 cited in the application see the whole document ---	1-7
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
28 November 1997	16. 12. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chakravarty, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DURIE F H ET AL.: "Antibody to the ligand of CD40, gp39, blocks the occurence of the acute and chronic forms of graft-vs-host disease." J. CLIN. INVEST., vol. 94, no. 3, September 1994, pages 1333-1338, XP002048597 cited in the application see the whole document ---	1-7
Y	WO 95 06666 A (DARTMOUTH COLLEGE ;NOELLE RANDOLPH J (US); FOY TERESA M (US)) 9 March 1995 cited in the application see the whole document ---	1-7
A	YANG Y ET AL: "TRANSIENT IMMUNE BLOCKADE PREVENTS FORMATION OF NEUTRALIZING ANTIBODY TO RECOMBINANT ADENOVIRUS AND ALLOWS REPEATED GENE TRANSFER TO MOUSE LIVER" GENE THERAPY, vol. 3, 1 January 1996, pages 412-420, XP000576295 see the whole document ---	
P,X	SCARIA A ET AL. : "Antibody to CD40 ligand inhibits both humoral and cellular immune responses to adenoviral vectors and facilitates repeated administration to mouse airway." GENE THERAPY, vol. 4, no. 6, June 1997, ENGLAND, pages 611-617, XP002048598 see the whole document ---	1-7
P,X	YANG Y ET AL: "Immunology of gene therapy with adenoviral vectors in mouse skeletal muscle." HUMAN MOLECULAR GENETICS, vol. 5, no. 11, November 1996, pages 1703-1712, XP002048599 see the whole document ---	1-7
P,X	YANG Y ET AL.: "Transient subversion of CD40 ligand function diminishes immune responses to adenovirus vectors in mouse liver and lung tissues." J. VIROL., vol. 70, no. 9, September 1996, pages 6370-6377, XP002048600 cited in the application see the whole document ---	1-7

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1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16487

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KAY MA ET AL.: "Transient immunomodulation with anti-CD40 ligand antibody and CTLA4Ig enhances persistence and secondary adenovirus-mediated gene transfer into mouse liver." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 94, no. 9, April 1997, WASHINGTON US, pages 4686-4691, XP002048601 see the whole document -----	1-7

1

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/16487

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-7
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/16487

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9506666 A	09-03-95	AU 3518397 A	30-10-97
		AU 7642994 A	22-03-95
		AU 678532 B	29-05-97
		AU 7644594 A	22-03-95
		CN 1134113 A	23-10-96
		EP 0721469 A	17-07-96
		EP 0721346 A	17-07-96
		FI 960978 A	29-04-96
		FI 960980 A	30-04-96
		HU 74251 A	28-11-96
		HU 74246 A	28-11-96
		HU 74250 A	28-11-96
		JP 9502096 T	04-03-97
		JP 9502186 T	04-03-97
		NO 960861 A	30-04-96
		NO 960862 A	30-04-96
		NZ 273207 A	22-09-97
		WO 9506481 A	09-03-95
		ZA 9406766 A	19-04-95
		AU 7643094 A	22-03-95
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		EP 0742721 A	20-11-96
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		JP 9502184 T	04-03-97
		NO 960863 A	30-04-96
		WO 9506480 A	09-03-95

CORRECTED VERSION*

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(30) Priority Data:
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(60) Parent Application or Grant
(63) Related by Continuation
US Not furnished (CON)
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(71) Applicant *(for all designated States except US)*: GENZYME CORPORATION [US/US]; One Moutain Road, Framingham, MA 01701-9322 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SCARIA, Abraham [IN/US]; 2 Foothill Road, Framingham, MA 01702 (US). KAPLAN, Johanne, M. [CA/US]; 78 Ivy lane, Sherborn, MA 01702 (US).

(74) Agent: SEIDE, Rochelle, K.; Brumbaugh, Graves, Donohue & Raymond, 30 Rockefeller Plaza, New York, NY 10112 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: INHIBITION OF PRIMARY AND/OR SECONDARY IMMUNE RESPONSE TO REPEAT ADENOVIRAL VECTOR ADMINISTRATION USING CD40L SPECIFIC ANTIBODIES

(57) Abstract

A method is provided for decreasing or eliminating an immune response to the administration of an adenoviral vector containing a transgene to an individual comprising co-administering to said individual an amount of an antibody specific for CD40L on CD4+ T lymphocytes such that primary and/or secondary immune responses to the adenoviral vector are diminished or eliminated and expression of the transgene persists.

*(Referred to in PCT Gazette No. 31/1998, Section II)

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Description

INHIBITION OF PRIMARY AND/OR SECONDARY IMMUNE RESPONSE TO REPEAT ADENOVIRAL VECTOR ADMINISTRATION USING CD40L SPECIFIC ANTIBODIES

Field of the Invention

The present invention relates to the use of antibodies specific for a helper T cell (T_h) ligand (CD40L), that binds to a receptor (CD40) that is found on B cells and other anigen presenting cells (APCs) and which is required for their activation. The antibodies of the invention are useful in reducing the host immune response to recombinant Adenoviral (Ad) vectors carrying a therapeutic transgene, thereby allowing effective repeat administration of such Ad vectors to a host.

Background of the Invention

E1-deleted replication-defective adenoviral (Ad) vectors are attractive vehicles for gene transfer to host cells because of their ability to transduce a wide variety of dividing and non-dividing cells *in vivo* (Stratford-Perricaudet et al., Hum. Gene Ther. 1:241-256 (1990); Rosenfeld et al., Cell 68:143-155 (1992); Zabner et al., Cell 75:207-216 (1993); Crystal et al., Nat. Genetics 8:42-51 (1994); Zabner et al., Nat. Genetics 6:75-83 (1994)). Such vectors have been used for transfer of the gene encoding normal human cystic fibrosis transmembrane conductance regulator (CFTR) into airway epithelial cells of experimental animals (e.g. mice, cotton rats, monkeys) and to airway epithelium of individuals with cystic fibrosis (CF) (Rosenfeld et al., Cell 68:143-155 (1992); Zabner et al., Cell 75:207-216 (1993); Crystal et al., Nat. Genetics 8:42-51 (1994); Zabner et al., Nat. Genetics 6:75-83 (1994)). Such vectors have transiently produced normal chloride ion channel function in CF patient airway epithelial cells.

A number of studies, however, have suggested that administration of high doses of first generation Ad vectors results in only transient CFTR gene expression in the lung due, at least in part, to destruction of vector-transduced cells by host cellular immune

responses (predominantly $CD8^{-}$ cytotoxic T cells) directed against Ad viral proteins and/or immunogenic transgene products (Yang et al., J. Virol. 69, 2004-2015 (1995); Kaplan et al., Gene Ther. 3:117-127 (1996); Tripathy et al., Nat. Medicine 2:545-550 (1996); Yang et al., Gene Ther. 3:137-144 (1996)). Reduction of this adverse immune response has been reported with the use of second generation vectors having decreased viral gene expression (Yang et al., Nature Genet. 7:362-369 (1994); Engelhardt et al., Proc. Natl. Acad. Sci. USA 91:6196-6200 (1994)) and with transgenes encoding self rather than foreign proteins (Tripathy et al., Nat. Medicine 2:545-550 (1996)).

The treatment of chronic diseases like CF with Ad vectors will likely require repeated administrations of Ad vectors containing the CFTR gene throughout the lifetime of the patient. However, as noted, the effectiveness of current Ad vectors is limited by the difficulty in obtaining successful readministration to an individual using a vector of the same Ad serotype, because of adverse immunologic responses. Various groups have demonstrated that a strong dose-dependent humoral immune response is induced by Ad vectors leading to the development of Ad-specific neutralizing antibodies, which leads to the inactivation by the host of readministered vector. (Yang et al., J. Virol. 69:2004-2015 (1995); Kaplan et al., Gene Ther. 3:117-127 (1996); Smith et al., Gene Ther. 5:397-402 (1993); Yei et al., Gene Ther. 1:192-200 (1994); Van Ginkel et al., Human Gene Ther. 6:895-903 (1995); Mastrangeli et al., Hum. Gene Ther. 7:79-87 (1996)). Studies using immunodeficient mice have shown that this process is dependent on MHC class II presentation of the input viral proteins and activation of $CD4^{+}$ T (helper) cells and can be induced by inactive as well as active viral particles (Yang et al., J. Virol. 69:2004-2015 (1995)).

In order to overcome the immunologic problems associated with repeat administration of Ad vectors, the use of broad immunosuppressants (Engelhardt et al., Proc. Natl. Acad. Sci. USA 91:6196-6200 (1994)) and cytoablative agents (Dai et al., Proc. Natl. Acad. Sci. USA 92:1401-1405 (1995)) to overcome the immune response of the host to first generation Ad vectors have been tested. Transient co-administration of an immunoglobulin, CTLA4-Ig, along with an intravenous injection of Ad vector

expressing a nonimmunogenic transgene product (human α-1 anti-trypsin) has been shown to lead to persistent transgene expression from mouse liver (Kay et al., Nat. Genetics 11:191-197 (1995)). CTLA4-Ig blocks the B7-CD28 pathway of T cell co-stimulation, which is required for optional activation of T cells. (Jenkins et al., Immunity 1:443-446 (1994); Lenschow et al., Ann. Rev. Immunol. 14:233-258 (1996)). Although Ad-specific antibody levels were reduced in CTLA4-Ig treated mice, the inhibition was not sufficient to allow secondary gene transfer via repeat administration of the vector under the conditions tested (Kay et al., Nat. Genetics 11:191-197 (1995)).

Co-administration of interferon-γ (INF-γ) or interleukin-12 (IL-12) with recombinant Ad vectors was shown to diminish the formation of Ad-specific neutralizing antibodies and allowed readministration of the vector to mouse airways (Yang et al., Nat. Medicine 1:890-893 (1995)). However, IL-12 is a potent mediator which affects T_{h1}-type $CD4^+$ T cell responses and is involved in stimulating natural killer (NK) cells and promoting the differentiation of cytotoxic T cells (CTLs) (Paul et al., Cell 76:241-251 (1994); Trinchieri, G., Blood 84:4008-4027 (1994); Bliss et al., J. Immunol. 156:887-894 (1996)). INF-γ is known to upregulate MHC class I on antigen presenting cells (Yang et al., Proc. Natl. Acad. Sci. USA 92:7257-7261 (1995)). Thus, both INF-Y and IL-12, while capable of inhibiting humoral immunity, might enhance the elimination of Ad vector transduced cells by CTLS (enhanced T_{h1} response).

It is well known that activated T cells play a critical role in the generation of both humoral and cellular immune responses. The interaction between the T cell receptor (TCR) and antigen-major histocompatibility complex (MHC) expressed on the surface of an antigen presenting cell (APC) is necessary, but not sufficient for the optimal activation of T cells which also requires additional co-stimulatory signals provided by several receptor-ligand pairs including B7-CD28 and CD40-CD40 ligand (CD40L) (Lenschow et al., Ann. Rev. Immunol. 14:233-258 (1996)).

CD40 is a 50kd molecule that has been identified on the surface of immature and mature B lymphocytes which, when crosslinked by antibodies, induces B cell proliferation. (Valle et al., Eur. J. Immunol. 19:1463-1467 (1989); Godon et al., J.

Immunol. 140:1425-1430 (1988); Gruber et al., J. Immunol. 142:4144-4152 (1989)). CD40, which has been found on human as well as murine B cells and other APCs, has been molecularly cloned and characterized. (Stamenkovic et al., EMBO J. 8:1403-1410 (1989)). Such other APCs include, inter alia, macrophages, dendritic cells, langerhans cells, endothelial cells, basal epithelial cells and thymic cells. (See, e.g., Foy et al., Ann. Rev. Immunol. 14; 591-617 (1996)).

A ligand for CD40, gp39 (also called CD40 ligand or CD40L) has also been molecularly cloned and characterized. (Armitage et al., Nature 357:80-82 (1992); Lederman et al., J. Exp. Med. 175:1091-1101 (1992); Hollenbaugh et al., EMBO J. 11:4313-4319 (1992)). CD40L (gp39) protein is expressed on activated, but not resting, $CD4^+$ T_h cells from humans and mice. (Spriggs et al., J. Exp. Med. 176:1543-1550 (1992); Lane et al., Eur. J. Immunol. 22:2573-2578 (1992); Roy et al., J. Immunol. 151:1-14 (1993)).

As noted, CD40L (gp39) has been shown to be expressed transiently at high levels on activated $CD4^+$ T cells (Noelle et al., Proc. Natl. Acad. Sci. USA 89:6550-6554 (1992); Foy et al., J. Exp. Med. 178:1567-1575 (1993)). The co-stimulation provided by CD40L on T cells interacting with CD40 on B cells and other APCs seems to be essential for thymus-dependent humoral immunity (Foy et al., J. Exp. Med. 178:1567-1575 (1993); Kawabe et al., Immunity 1:167-178 (1994); Xu et al., Immunity 1:423-431 (1994); Foy et al., Ann. Rev. Immunol. 14:591-617 (1996)). For example, cells transfected with the CD40L (gp39) gene and expressing CD40L on their surface can trigger B cell proliferation and, together with other stimulatory signals, can induce antibody production. (Armitage et al., Nature 375:80-82 (1992); Hollenbaugh et al., EMBO J. 11:4313-4319 (1992)). CD40L also appears to play an important role in the generation of cellular immune responses as a component of the cascade of events leading to the production of helper cytokines (Foy et al., Ann. Rev. Immunol. 14:591-617 (1996); Stuber et al., J. Exp. Med. 183:693-698 (1996); Stout et al., J. Immunol. 156:8-11 (1996)).

It has been shown that blockage of CD40L, e.g. with a specific antibody to CD40L can block the occurrence of chronic and acute forms of graft vs. host (GVH) disease (Durie et al., J. Clin. Invst. 94:1333-1338 (1994) and prevent collagen induced arthritis (Durie et al., Science 261:1328-1330 (1993). Likewise, published PCT application WO95/06666 discloses various anti-CD40L antibodies and their uses in mediating or inhibiting various helper T cell mediated immune responses. Moreover, transient subversion of CD40L by a specific antibody to CD40L has been shown to diminish primary immune responses to Ad vectors in mouse liver and lung tissues (Yang et al., J. Virol 70:6370-6377 (1996)).

Summary of the Invention

The present invention is directed to reducing the immune response of a host to administered adenoviral (Ad) vectors carrying a therapeutic transgene, thereby allowing for repeat readministration of such vectors to the host without (or with minimized) adverse immune responses.

The present invention is of particular relevance since Ad vectors are especially attractive for use in delivering a therapeutic transgene to host cells, e.g., in gene therapy, based in part on their ability to efficiently transfer the transgene into host cells, particularly non-dividing cells, in vivo. However, significant immune response to such Ad vectors, inflammation and loss of transgene expression has limited the effective use of Ad vectors in gene transfer and therapy.

The immune response to Ad vectors appears to be mediated through activation of $CD4^+$ T cells by viral antigens leading to $CD8^+$ cytotoxic T lymphocyte (CTL) and humoral immune responses to the vector. The CD40 ligand (CD40L) on T cells and CD40 receptors on B cells and other APCs are involved in generating the adverse immune response to administered Ad vectors.

The present invention thus provides for co-administration of monoclonal antibodies (MAbs) specific for CD40L together with recombinant Ad vectors in order to

minimize or ablate the host immune response to the administered Ad vector, thereby allowing for repeat administration of such vectors and persistent transgene expression.

Specifically, in a mouse model system, MAb to murine CD40L provided for transient blockade of co-stimulation between activated T cells ($CD4^-$) and B cells/antigen presenting cells (APCs) and inhibited neutralizing antibodies and the cellular response to a co-administered Ad vector. Co-administered anti-CD40L MAb also provided for increased persistence of expression of the transgene delivered to host cells by the Ad vector.

In a further aspect of the invention, the administration of anti-CD40L MAb interfered with secondary immune (antibody) responses in a preimmunized host to readministered Ad vectors, thereby allowing for repeat administrations of the vector, with high levels of transgene expression. This is highly significant, because most human recipients of Ad vector - based gene therapy are expected to have prior exposure to human adenoviruses and, therefore, to have an immunological memory directed to adenovial antigens.

In a still further aspect of the invention, the anti-CD40L MAb is a specific MAb designated MR1, which is a hamster-derived anti-murine CD40L MAb. The expressed transgene is the DNA for CFTR or β-galactosidase.

Brief Description of the Drawings

The present invention may be further understood with reference to the attached drawings, of which

Fig. 1 shows the effect of MR1 on the development of Ad-specific antibodies following administration of Ad2/CFTR2 vector. Panel A shows Ad-specific serum antibodies. Panel B shows Ad-specific IgA levels in the lung.

Fig. 2 shows the effect of MR1 on efficient readministration of a second dose of an Ad vector. The graphs show β-galactosidase expression in the presence and absence of administered MR1.

Fig. 3 depicts the effect of MR1 on CTL responses in mice intranasally administered Ad2/βGal4.

Fig. 4 shows the effect of MR1 on persistence of transgene (β-gal) expression in mice.

Fig. 5 shows decreased lung inflammation to Ad2/CFTR2 in MR1-treated mice.

Fig. 6 shows the effect of MR1 administration on the secondary immune response to Ad vectors.

Fig. 7 shows the effect of MR1 administration on Ad-specific antibody neutralizing titers upon readministration of Ad vectors.

Fig. 8 shows the effects of MR1 administration on transgene expression (β-gal) upon a third administration of Ad vector.

Detailed Description of the Invention

The present invention is directed to diminishing or inhibiting adverse immunologic responses in a host individual to an administered Adenoviral (Ad) vector comprising a therapeutic transgene. The invention involves co-administering to said host individual an effective amount of an inhibitor for CD40L on $CD4^{+}$T cells, particularly an antibody, and more particularly a monoclonal antibody (MAb) specific for CD40L. The dosage, timing, and routes of administering the Ad vector and CD40L specific MAb to the host is chosen to be most effective in minimizing or inhibiting the primary and secondary humoral and cellular immune responses in the host to the administered vector. Thus, Ad-specific neutralizing antibodies, CTLs and inflammatory responses are reduced. The administration of CD40L specific antibodies allows for repeat administration of the same adenoviral vectors to the host, with minimized adverse immunological consequences and persistent transgene expression in treated host cells.

In preferred aspects of the invention, the adenoviral (Ad) vector is an Ad 2 vector having a substantially deleted E1 region and E4 region (except for open reading frame 6). The vector further comprises a therapeutic transgene operably linked to expression control sequences (promoter, poly A-tail) inserted into the deleted E1 region of the

vector. In a preferred example, the vector is Ad2/CFTR2 which contains the DNA coding for cystic fibrosis transmembrane regulator and can. upon administration to airway epithelial cells of CF patients, result in functional chloride ion channel activity in such cells. (Zabner et al., J. Clin. Invest. 97:1504-1511 (1996)).

Other vectors useful in the present invention include Ad2/β-Gal2 and Ad2/β-Gal4. These latter Ad vectors express β-gal in host cells which can be used as a marker for transgene expression. Other Ad vectors comprising additional therapeutic transgenes are within the scope of the invention.

In other aspects of the invention, the effectiveness of the approach to reducing adverse immune responses to Ad vectors is demonstrated in a standard mouse model system, particularly Balb/c mice. In particular, the Ad vectors used in the invention are intranasally administered to the mice, resulting in Ad infection of host airway epithelial cells (e.g. in the lung).

In order to reduce or inhibit primary and secondary immune responses to the administered Ad vector and provide for effective readministration of the vector and persistent transgene expression, CD40L on $CD4^+$T cells was blockaded by co-administration of anti-murine CD40L MAb, particularly the hamster-derived MR1 antibody. While whole purified MR1 antibody was used, it is expected that fragments of such antibodies, e.g. $(Fab')_2$, Fab, Fv and others should also be useful in producing the desired response.

Moreover, while effectiveness of the approach to reducing the immune response to Ad vectors via inhibiting CD40L has been shown in a mouse model system, it is expected that such approaches also work in humans, particularly CF patients. It has already been demonstrated that CFTR containing Ad vectors can produce functional chloride channels in airway epithelium in CF patients. The co-administration of anti-human CD40L antibodies can reduce or inhibit the adverse immune responses believed to be the primary reason for reduced effectiveness of repeated CFTR-containing Ad vector administration for treating CF patients.

The anti-human CD40L antibodies for inhibiting inmmune responses to Ad vectors in humans can be either polyclononal or monocional antibodies (MAbs). Such antibodies can be of animal origin such as rabbit or rodent MAbs. The anti-human DC40L antibodies can be of human origin. Such antibodies also can be of animal origin. e.g. rodent MAbs. that have been "humanized" using techniques known by those skilled in the art.

Example 1

Anti CD40L Monoclonal Antibodies

Anti-CD40L MAbs may be made as known in the art. A detailed recitation of methods for producing such antibodies is provided in published PCT application WO95/06666. incorporated herein by reference. Techniques can be used to produce such MAbs in mice. hamsters and rabbits. Likewise. human MAbs to CD40L may be obtained as described in WO95/06666.

MR1, a hamster anti-mouse CD40L was produced in ascites fluid and purified by ion exchange HPLC as described by Noelle et al., Proc. Natl. Acad. Sci. USA 89:6550-6554 (1992). incorporated herein by reference.

Other antibodies contemplated for use in the present invention include anti-human CD40L MAbs of murine origin as disclosed in WO95/06666 (Example 6). Such antibodies include. inter alia. the hybridomas designated 89-76 and 24-31 that have been deposited with the ATCC.

However. for administration of anti CD40L antibodies to humans. it may be preferable to use antibodies of human origin or those that have been "humanized" to avoid or reduce potential adverse immunological responses to rodent MAbs (e.g. mouse or hamster). It is believed. however. that any such potential adverse effects should be minimal. since the reason for administering antibodies to CD40L is to reduce or inhibit immune responses to a foreign antigen. Techniques known to those of skill in the art. e.g.. recombinant technology may be used to construct "humanized" anti-CD40L antibodies that maintain the high binding affinity of the rodent MAbs.

For example, recent approaches to humanizing rodent MAbs use only the rodent complementarity determining regions (CDRs), rather than the entire V domain, transplanted to a human antibody. Such humanized antibodies are known as CDR-grafted antibodies. CDRs are regions of hypervariability in the V regions that are flanked by relatively conserved regions known as framework (FR) regions. Each V domain contains three CDRs flanked by four FRs. The CDRs fold to form the antigen binding site of the antibody, while the FRs support the structural conformations of the V domains. Thus by transplanting the rodent CDRs to a human antibody, the antigen binding domain theoretically also is transferred. (Owens et al. J. Immunol. Methods 168:149 (1994) and Winter et al. Immunology Today 14:243 (1993) incorporated herein by reference.)

Different avidities of humanized MAbs also appear to depend upon the particular human framework region (FR) of the humanized antibody. For example, Co et al. Proc. Natl. Acad. Sci. USA 88:2869 (1991) required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. Protein Engineering 4:773 (1991) reported the influence of particular FR residues of a CDR-grafted antibody on antigen binding, and proposed that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. J. Immunol. 150:2844 (1993) reported that optimal humanization of an anti-CD18 murine monoclonal antibody was dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation.

Accordingly, it will be apparent to those skilled in the art that recreation of the antigen-binding site requires consideration of the potential intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see e.g., Owens et al.), in all cases the procedures must be tailored and optimized for the particular rodent antibody of interest.

Example 2

Antibody Injections

Balb/c mice, purchased from Taconic Laboratories (Germantown. NY) were used as the model system for assessing the effectiveness of anti-CD40L MAbs in reducing immune responses to administered Ad vectors.

Typically. mice were injected intraperitoneally (IP) with a total of four injections each of 200-250 μg purified antibody (MR1) starting on day -2 relative to the time of administering the Ad vectors.

Example 3

Adenoviral Vectors

The construction of Ad2/CFTR2 has been described in detail in allowed U.S. Patent Application Serial No. 08/136,742 and in Armentano et al. (1995) Human Gene Ther. 6:1353. both incorporated herein by reference.

Ad2/CFTR2. is an Ad2 based vector in which substantially all of the adenoviral E1 region has been deleted and replaced with a CFTR transgene expression cassette comprising a PGK promoter. the CFTR encoding sequence and a BGH poly A site and most of the E4 region has been deleted. except for open reading frame 6 (ORF-6).

Administration of Ad2CFTR2 to nasal epithelia of CF patients has resulted in restoration of a functional chloride ion channel in treated cells. Zabner et al. J. Clin. Invst. 97:1504-1511 (1996) incorporated herein by reference. However, adverse host immune responses limited the gene transfer effectiveness.

Ad2/βGal2 is derived from Ad2/CFTR2, but with the CFTR expression cassette replaced by a DNA fragment comprising the CMV promoter. the lacZ(β-galactosidase encoding) gene and a 5' nuclear localization signal from the SV40T antigen.

Ad2/βGal4 is similar to Ad2/βGal2 but it contains a complete wild type E4 region rather than just ORF6.

Example 4

Histopathology of Mouse Lung
Tissue Following Ad Vector Administration

On the day of sacrifice, mice were euthanized with an IP injection of Somlethal. The lungs were cleared of blood by vascular perfusion with phosphate buffered saline (PBS). The trachea was cannulated and the lungs and trachea removed. The lungs were fixed by inflation with 2% paraformaldehyde containing 0.2% glutaraldehyde in PBS, pH 7.4, at a pressure of 30 cm of H_20. Following overnight fixation, portions of the left lung were embedded in glycomethacrylate, sectioned and stained with hematoxylin and eosin. These sections were evaluated by light microscopy for the presence and distribution of lung inflammation without previous knowledge of treatment as provided in Ginsberg et al. Proc. Natl. Acad. Sci. USA 88:1651-1655 (1991), incorporated herein by reference. The lung sections were subjectively assessed for morphologic alterations on a scale of 0-4: 0=no lesion, 1=minimal, 2=mild, 3=moderate, 4=severe.

Example 5

Measurement of Host Antibodies to Adenovirus

Titers of Ad-specific serum antibodies were evaluated by ELISA techniques as follows. Serial 2-fold dilutions of sample were added to the wells of a 96 well plate coated with photochemically inactivated Ad2 (Lee Biomolecular Research, San Diego, CA). Bound virus-specific antibodies were detected by the addition of horseradish peroxidase (HRP)-conjugated goat anti-mouse Ig (IgG, IgM, IgA-specific; Jackson Immunoresearch Laboratories, West Grove, PA). The titer was defined as the reciprocal of the highest dilution of sample which produced an $O.D._{490}$ greater than 0.1.

To evaluate levels of Ad-specific IgA in bronchoalveolar lavages (BAL), samples were diluted 2-fold and added to Ad2-coated plates followed by the addition of HRP-conjugated goat anti-mouse IgA (α chain-specific; Cappel, Durham, NC). For quantitation, a standard curve was constructed using a monoclonal antibody against mouse IgA (Harlan Sera-Lab, Sussex, England) to coat ELISA plates and capture known amounts of purified mouse IgA (Cappel). The $O.D._{490}$ values obtained following the

addition of HRP-conjugated goat antimouse IgA were plotted against the amounts of IgA standard (ng/ml) added to the wells. The concentrations of Ad-specific IgA present in BAL samples were then derived from the standard curve by linear regression analysis.

Example 6

Evaluation of Cytoxic T Lymphocyte (CTL)
Activity in Response to Adenoviral Vectors

To evaluate cytotoxic T lymphocyte (CTL) activity, spleen cells from animals in the same group were pooled and stimulated in vitro with mitomycin-C inactivated, syngeneic fibroblasts infected with Ad2/βGal-4 at a multiplicity of infection (M.O.I.) of 50 for 24 hr. Cells were cultured in 24-well plates containing 5×10^6 spleen cells and 6×10^4 stimulator fibroblasts per well in a 2 ml volume. The culture medium consisted of RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 5×10^{-5}M 2-mercaptoethanol, 20 mM HEPES buffer and 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT). Cytolytic activity was assayed after 5-7 days of culture.

Target fibroblasts were infected with Ad vector at an MOI of 100 for 48h and were treated with 100 U/ml recombinant mouse γ-interferon (Genzyme, Cambridge, MA) for approximately 24 hr before use to enhance MHC Class I expression and antigen presentation to effector CTLS. The fibroblasts were labeled with ^{51}Chromium (^{51}Cr; NEN) overnight (50 μCi/10^5 cells) and added to the wells of a round-bottom 96 well plate in a 100 μl volume (5×10^3 fibroblasts/well). Effector cells were added in a 100 μl volume at various effector:target cell ratios in triplicate. After 5 hours of incubation at 37°C/5% CO_2, 100 μl of cell-free supernatant was collected from each well and counted in a Packard (Downers Grove, IL) Multi-Prias gamma counter. The amount of ^{51}Cr spontaneously released was obtained by incubating target fibroblasts in medium alone and the total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water. The percentage lysis was calculated as follows:

$$\% \text{ Lysis} = \frac{(\text{Sample cpm}) - (\text{Spontaneous cpm})}{(\text{Total cpm}) - (\text{Spontaneous cpm})} \times 100$$

Example 7

Measurement of β-Galactosidase Expression

For quantitation of β-gal expression, lungs from individual mice were homogenized and assayed using the AMPGD kit obtained from Tropix, Bedford, MA. The protein concentration in an individual sample was determined using the BioRad DC reagent (BioRad, Hercules, CA) and the results are expressed as relative light units (RLU)/μg protein.

Example 8

Determination of Titers of Neutralizing Antibodies Against Adenoviral Vectors

To determine Ad specific neutralizing antibody titers, serial 2-fold dilutions of an antibody sample were incubated with live Ad2/CFTR-2 for 1 hour at 37°C/5% CO_2 in the wells of flat bottom 96 well plates. At the end of the incubation period, permissive 293 cells were added to the wells and the plates were incubated at 37°C/5% CO_2 for 72-96 hours. The assay was read when control 293 cells incubated alone reached ≥90% confluency. The neutralizing antibody titer was defined as the reciprocal of the highest dilution of sample that showed any detectable protection of 293 cells from cytopathic effects when compared to cells incubated with untreated virus or virus incubated with seronegative serum.

Example 9

Monoclonal Antibody Specific For CD40L Inhibits Development of Host Antibodies to Administered Adenoviral Vectors

To explore the role of the CD40-CD40L interaction in the generation of antibodies to Ad vectors, Balb/c mice were injected intraperitoneally with the anti-CD40L MAb MRI (Example 1). 200 μg/injection/mouse of MR1 on days -2, +2, +6 and +10) was given (Example 2). Ad2/CFTR2 vector (10^9 I.U.) was instilled intranasally on

day 0. Ad specific antibodies were measured as in Example 5. Analysis of serum from these animals by ELISA showed a marked decrease in anti-Ad antibody (IgG + IgM + IgA) titers in MRI treated mice for up to 41 days. Fig. 1A is a graph plotting Ad specific serum antibody titers measured by ELISA. Closed circles (-•-) show Anti-Ad titers in mice treated with MR1; open squares (-□-) show those not treated with MR1. Each point represents the mean titer of groups of individual mice (n=4) ± standard error of the mean (SEM).

Analysis of bronchoalveolar lavage (BAL) fluid 40 days after administration of the vector analyzed by ELISA revealed a parallel drop in Ad specific IgA levels in MRI treated mice (Figure 1B). The data are mean IgA levels (n=3) ±SEM. (Fig. 1B).

On day 38 after the first administration of Ad2/CFTR2, a vector of the same serotype, Ad2/βGal2, was administered to the different groups of mice. Expression of β-galactosidase was measured by the quantitative assay of Example 7 on day 41. The data, which are presented as mean ± SEM (n=4) shows elevated β-galactosidase levels in MRI treated mice compared to control mice (Fig. 2).

Example 10

Monoclonal Antibody to CD40L Produces
A Decreased CTL Response to Administered
Adenoviral Vector and Results in Increased
Persistence of Transgene Expression

BALB/c mice were instilled intranasally with 10^9 IU of Ad2/βGal-4 on day 0 and injected with MRI (250 μg/mouse/injection) on days -2, +2, +5 and +8. The spleen cells were collected on day 21, re-stimulated in vitro with infected syngeneic fibroblasts and tested for cytolytic activity. The results shown in Fig. 3 are mean percent lysis from triplicate wells at various target:effector ratios. Spleen cells from MR1 treated mice showed decreased yet measurable levels of CTL activity compared to spleen cells from untreated control mice, albeit using an assay that is not strictly quantitative (Example 6).

Since CTLs have been implicated in loss of transgene expression, administration of MR1 with an Ad vector containing β-gal transgene was tested to determine whether

the MR1 MAb would give rise to prolonged transgene expression. As shown in Fig. 4, transgene expression measured by the quantitative galactosidase assay of Example 7, declined to background levels by day 21 in the untreated controls. By comparison, in MR1 treated mice, transgene expression also declined but remained consistently higher than in untreated mice. The data are presented as mean ±SEM(n=4).

Example 11

Monoclonal Antibody to CD40L Results in
Decreased Inflammatory Responses to Adenoviral Vectors

Lung tissue from the mice of Example 9 was examined as provided in Example 4 for evidence of histopathological changes in the peribronchial, perivascular and alveolar regions. Lung inflammation characterized by inflammatory cell infiltrates was present on day 5 and was not yet resolved at day 21. On day 5, there were no differences noted between the lungs of mice treated with the vector either with or without MR1 antibody treatment (data not shown). However, as shown in Fig. 5, on day 21, there were fewer inflammatory changes in all regions of the lungs from mice treated with the MR1 antibody. The inflammatory cell infiltrate was markedly reduced in the peribronchial/ peribronchiolar and perivascular regions.

Example 12

Monoclonal Antibody to CD40L Inhibits
Secondary Antibody Response to Adenoviral
Vectors in Preimmunized Hosts

MR1 was tested for its ability to interfere with the secondary antibody response in mice that had been preimmunized with an Ad vector. Mice were intranasally instilled with 10^8 IU of Ad2/CFTR2 on day 0. On day 50, 10^8 IU of Ad2/CFTR2 was intranasally administered to the same mice. It had previously been determined that an intranasal instillation of 10^8 IU of Ad vector elicits both humoral and cellular immune responses to the vector in several strains of mice including BALB/c (data not shown). MR1 injections were given around the time of the second virus administration on days 44, 48, 52 and 56.

Sera from the different groups of animals were analyzed both for anti-Ad ELISA titers (IgG + IgM + IgA) and Ad neutralizing titers at various time points (as provided in Example 5). Fig. 6 shows that the secondary antibody response to Ad measured by ELISA (serial 2-fold dilutions of sera obtained at the indicated times) was quite robust in both MR1-treated and -untreated mice and was only slightly decreased in the MR1-treated mice.

Ad neutralizing titers, on the other hand, were clearly decreased in the mice that had received MR1 along with the second administration of the Ad vector (Fig. 7). In addition, the decline in anti-Ad titers, after reaching a peak around day 84, was more rapid in MR1 treated mice compared to untreated mice (Fig. 6).

Example 13

Effect of Monoclonal Antibody to CD40L
On Third Administration of Adenoviral Vectors

A third intranasal administration of Ad2/βGal-2 vector was given on day 99 and β-galactosidase levels in the lung were measured on day 102. Control mice that had received the first (day 0) and second dose (day 50) of Ad2/CFTR2 showed only 6% of the β-galactosidase activity in lung homogenates compared to naive mice that received a single intranasal administration of Ad2/βGal-2 (Fig. 8). Mice that received MR1 around the time of the second administration of Ad2/CFTR2 showed 10-fold higher levels of transgene expression after the third administration compared to untreated control mice (Fig. 8). The effectiveness of the third vector administration in the MR1 treated animals was comparable to that obtained in naive mice.

Claims

1. A method for decreasing or eliminating an immune response to the administration of an adenoviral vector containing a transgene to an individual comprising co-administering to said individual an amount of an antibody specific for CD40L on CD4+ T lymphocytes such that primary and/or secondary immune responses to the adenoviral vector are diminished or eliminated and expression of the transgene persists.

2. The method of Claim 1, in which the antibody is a monoclonal antibody.

3. The method of Claim 2, in which the monoclonal antibody is MR1.

4. The method of Claim 2, in which the monoclonal antibody is selected from the group consisting of hybridoma 89-76 and hybridoma 24-31.

5. The method of Claim 1, in which the antibody is a fragment of an antibody.

6. The method of Claim 1, in which the antibody is a humanized antibody.

7. The method of Claim 1, in which the adenoviral vector is Ad2/CFTR2.

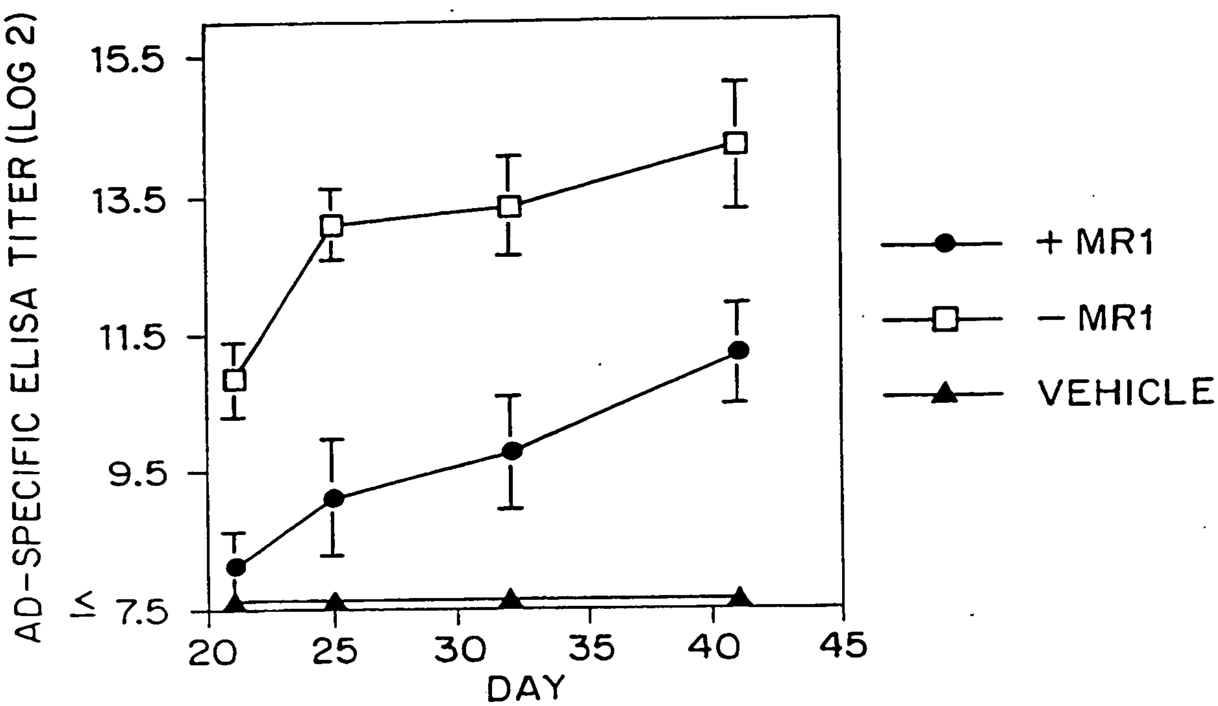

AD-SPECIFIC ELISA TITER (LOG 2)
15.5
13.5
11.5
9.5
≤ 7.5
20
25
30
35
40
45
DAY
+MR1
−MR1
VEHICLE

FIG. 1A

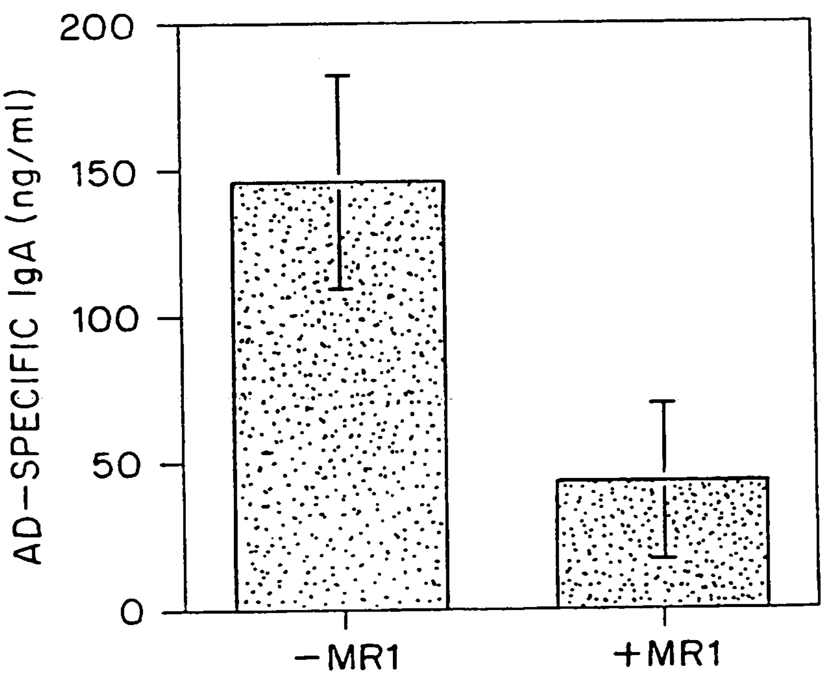

AD-SPECIFIC IgA (ng/ml)
200
150
100
50
0
−MR1
+MR1

FIG. 1B

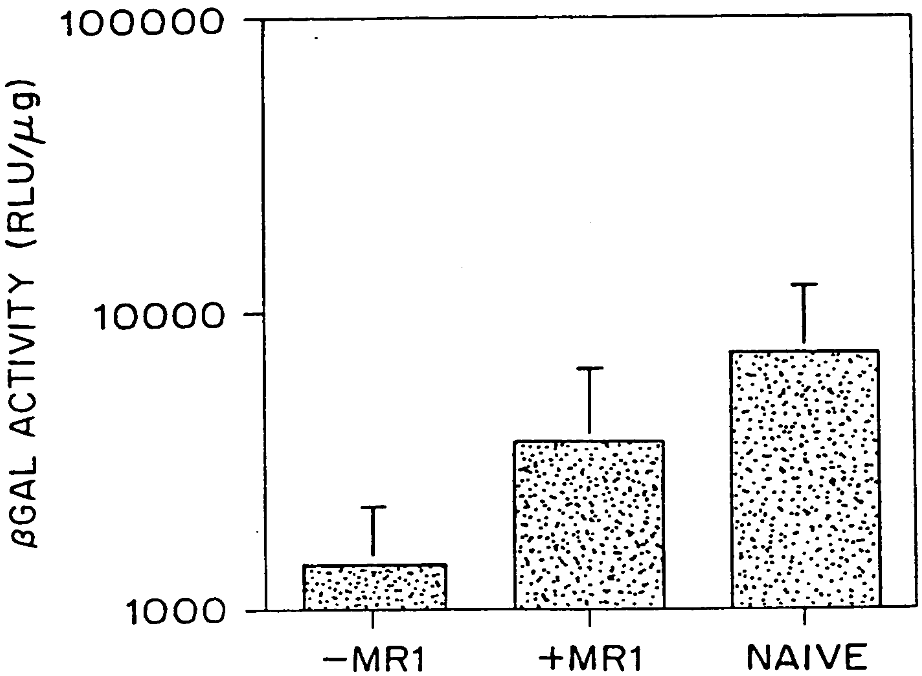

βGAL ACTIVITY (RLU/μg)
100000
10000
1000
−MR1
+MR1
NAIVE

FIG. 2

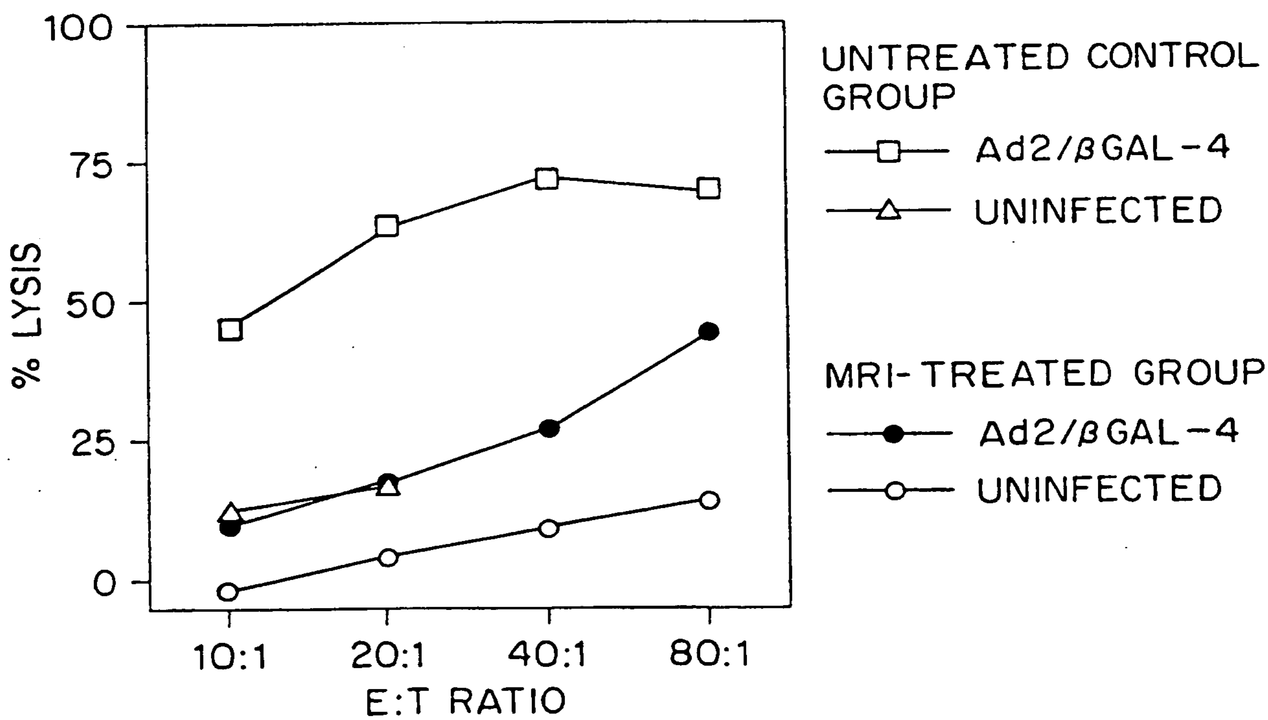

% LYSIS
100
75
50
25
0
10:1
20:1
40:1
80:1
E:T RATIO
UNTREATED CONTROL GROUP
Ad2/βGAL-4
UNINFECTED
MRI-TREATED GROUP
Ad2/βGAL-4
UNINFECTED

FIG. 3

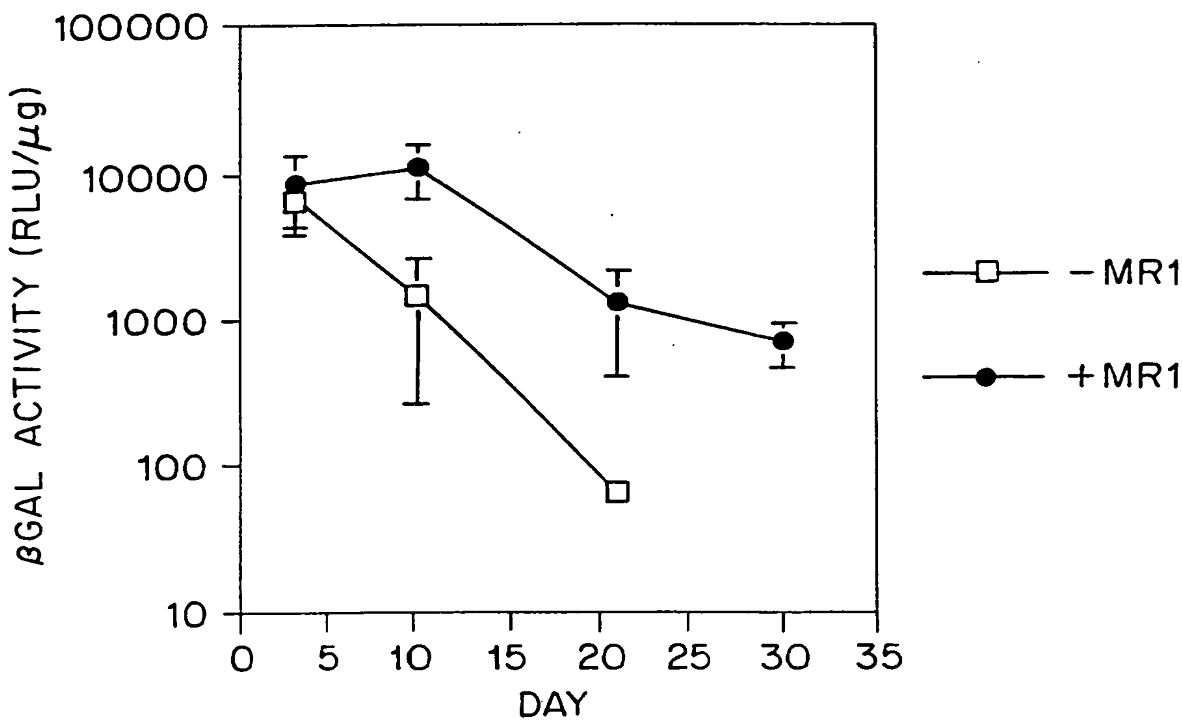

100000
10000
1000
100
10
βGAL ACTIVITY (RLU/μg)
0
5
10
15
20
25
30
35
DAY
−MR1
+MR1

FIG. 4

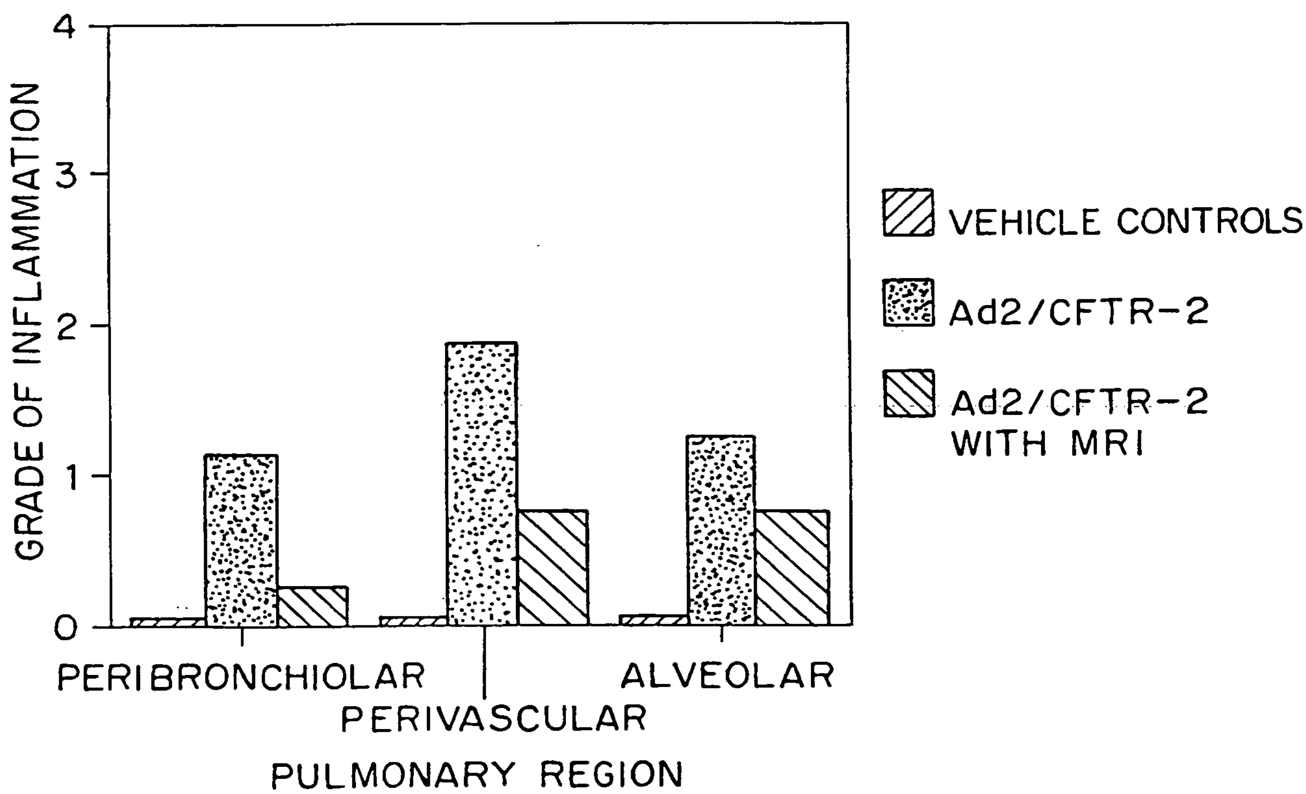

4
3
2
1
0
GRADE OF INFLAMMATION
VEHICLE CONTROLS
Ad2/CFTR−2
Ad2/CFTR−2
WITH MRI
PERIBRONCHIOLAR
PERIVASCULAR
ALVEOLAR
PULMONARY REGION

FIG. 5

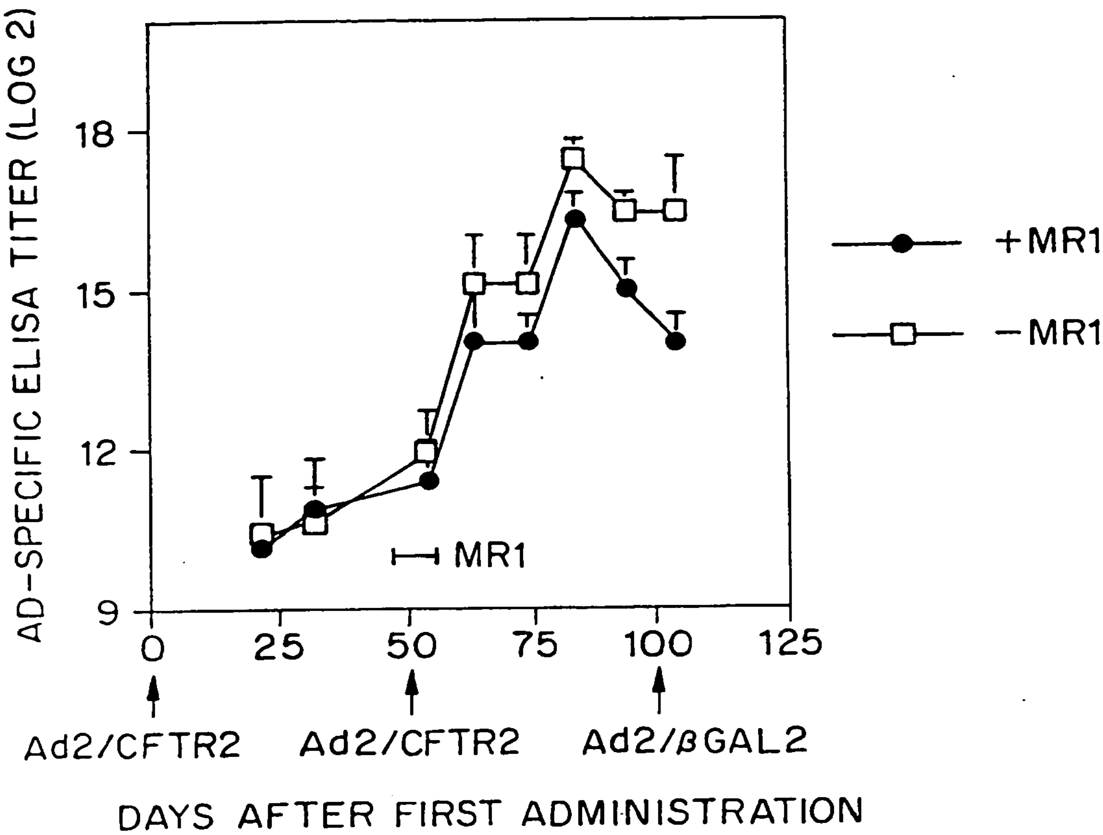

AD-SPECIFIC ELISA TITER (LOG 2)
18
15
12
9
0
25
50
75
100
125
MR1
+MR1
−MR1
Ad2/CFTR2
Ad2/CFTR2
Ad2/βGAL2
DAYS AFTER FIRST ADMINISTRATION

FIG. 6

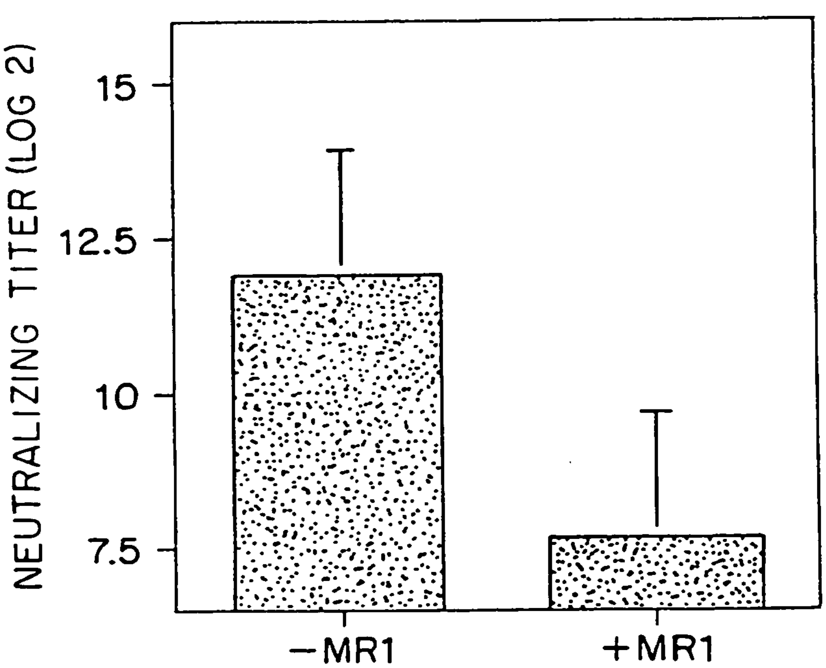
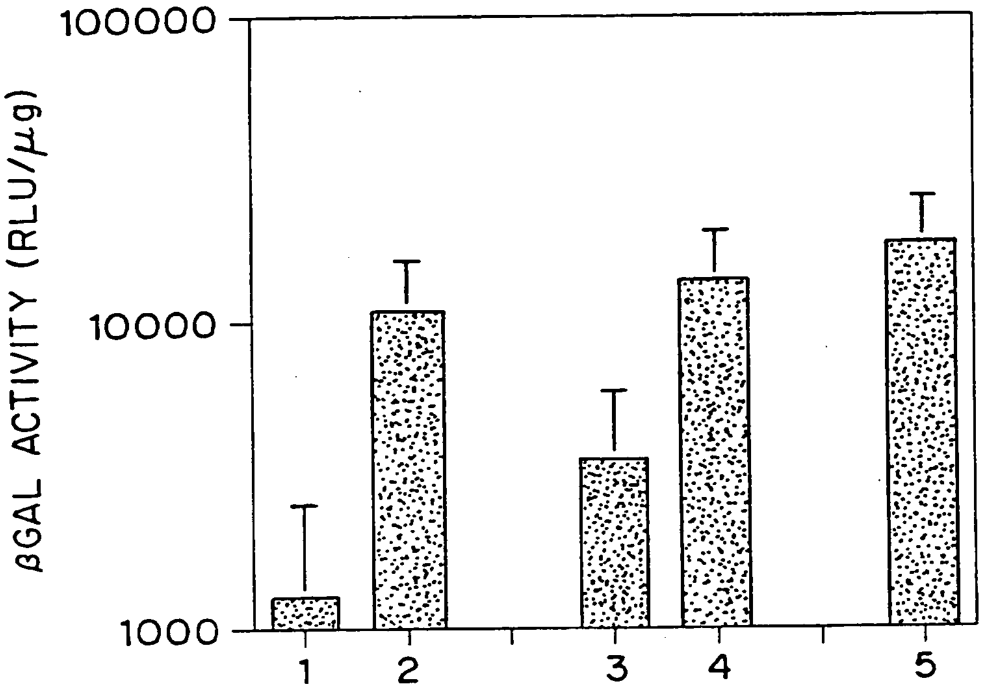

NEUTRALIZING TITER (LOG 2)
15
12.5
10
7.5
−MR1
+MR1

FIG. 7



GROUP	DAY 0	DAY 50	DAY 99
1	Ad2/CFTR2	Ad2/CFTR2	Ad2/βGAL2
2	Ad2/CFTR2	Ad2/CFTR2+MR1	Ad2/βGAL2
3	—	Ad2/CFTR2	Ad2/βGAL2
4	—	Ad2/CFTR2+MR1	Ad2/βGAL2
5	—	—	Ad2/βGAL2

FIG. 8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16487

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 C07K16/28 //A61K48/00,C12N15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DURIE F H ET AL: "PREVENTION OF COLLAGEN-INDUCED ARTHRITIS WITH AN ANTIBODY TO GP39, THE LIGAND FOR CD40" SCIENCE, vol. 261, no. 5126, 3 September 1993, pages 1328-1330, XP002035702 cited in the application see the whole document ---	1-7
Y	KAY M A ET AL: "Long-term hepatic adenovirus-mediated gene expression in mice following CTLA4Ig administration." NATURE GENETICS, vol. 11, no. 2, October 1995, UNITED STATES, pages 191-197, XP000676074 cited in the application see the whole document ---	1-7
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
28 November 1997	16. 12. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DURIE F H ET AL.: "Antibody to the ligand of CD40, gp39, blocks the occurence of the acute and chronic forms of graft-vs-host disease." J. CLIN. INVEST., vol. 94, no. 3, September 1994, pages 1333-1338, XP002048597 cited in the application see the whole document ---	1-7
Y	WO 95 06666 A (DARTMOUTH COLLEGE ;NOELLE RANDOLPH J (US); FOY TERESA M (US)) 9 March 1995 cited in the application see the whole document ---	1-7
A	YANG Y ET AL: "TRANSIENT IMMUNE BLOCKADE PREVENTS FORMATION OF NEUTRALIZING ANTIBODY TO RECOMBINANT ADENOVIRUS AND ALLOWS REPEATED GENE TRANSFER TO MOUSE LIVER" GENE THERAPY, vol. 3, 1 January 1996, pages 412-420, XP000576295 see the whole document ---	
P,X	SCARIA A ET AL. : "Antibody to CD40 ligand inhibits both humoral and cellular immune responses to adenoviral vectors and facilitates repeated administration to mouse airway." GENE THERAPY, vol. 4, no. 6, June 1997, ENGLAND, pages 611-617, XP002048598 see the whole document ---	1-7
P,X	YANG Y ET AL: "Immunology of gene therapy with adenoviral vectors in mouse skeletal muscle." HUMAN MOLECULAR GENETICS, vol. 5, no. 11, November 1996, pages 1703-1712, XP002048599 see the whole document ---	1-7
P,X	YANG Y ET AL.: "Transient subversion of CD40 ligand function diminishes immune responses to adenovirus vectors in mouse liver and lung tissues." J. VIROL., vol. 70, no. 9, September 1996, pages 6370-6377, XP002048600 cited in the application see the whole document ---	1-7
	-/--	

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KAY MA ET AL.: "Transient immunomodulation with anti-CD40 ligand antibody and CTLA4Ig enhances persistence and secondary adenovirus-mediated gene transfer into mouse liver." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 94, no. 9, April 1997, WASHINGTON US, pages 4686-4691, XP002048601 see the whole document -----	1-7

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/16487

B x I Observations where certain claims were found unsearchabl (Continuation f item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-7
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/16487

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9506666 A	09-03-95	AU 3518397 A	30-10-97
		AU 7642994 A	22-03-95
		AU 678532 B	29-05-97
		AU 7644594 A	22-03-95
		CN 1134113 A	23-10-96
		EP 0721469 A	17-07-96
		EP 0721346 A	17-07-96
		FI 960978 A	29-04-96
		FI 960980 A	30-04-96
		HU 74251 A	28-11-96
		HU 74246 A	28-11-96
		HU 74250 A	28-11-96
		JP 9502096 T	04-03-97
		JP 9502186 T	04-03-97
		NO 960861 A	30-04-96
		NO 960862 A	30-04-96
		NZ 273207 A	22-09-97
		WO 9506481 A	09-03-95
		ZA 9406766 A	19-04-95
		AU 7643094 A	22-03-95
		CN 1137758 A	11-12-96
		EP 0742721 A	20-11-96
		FI 960979 A	30-04-96
		JP 9502184 T	04-03-97
		NO 960863 A	30-04-96
		WO 9506480 A	09-03-95